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CHEMOTROPISM IN RHIZOPUS NIGRICANS[1]

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(WITH FOUR FIGURES)

Historical

In view of the important relation of the question of chemotropism among the fungi to the problems of fungal parasitism, it is surprising that investigations in this field have been so meager; for while numerous scattered observations may be found here and there in the literature, the works of MIYOSHI (**8**) and FULTON (**5**) stand out as the only ones which represent any considerable investigations of the subject. The researches of CLARK (**3**), although intended primarily to supplement his work on the toxicity of copper compounds, are also of especial importance in this connection.

It is needless to enter here into a detailed historical account of all the researches from which information can be culled regarding chemotropism in the fungi, for an excellent review of these may be found in FULTON'S paper. It is our purpose merely to outline the results obtained by MIYOSHI, CLARK, and FULTON.

MIYOSHI (**8**) tested a great variety of chemical substances, and maintained that in many cases they exerted a very marked attraction on the fungal hyphae, which then grew toward them (positive chemotropism); others showed a repellent effect, so that the hyphae grew diametrically away from the diffusion center (negative

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chemotropism); while still others produced no effect. Sugars were especially attractive to the molds.

The most important methods employed by MIYOSHI were as follows: (1) the substances to be tested were injected in solution into leaves, which were then sown with spores; or (2) the substances were incorporated in a gelatin layer, on which was laid a perforated mica plate or membrane, the spores being sown above, either on the free surface of the plate or membrane, or in a superimposed layer of medium lacking the substance to be tested; or (3) fine glass capillary tubes holding the substance to be tested were introduced into a preparation containing germ tubes of the fungus growing in water or in a very dilute nutrient solution.

Where the germ tubes showed a marked turning toward the stomata, perforations, or open ends of the capillary tubes, as the case might be, and thus toward the diffusion centers of the chemical substances, the turnings were interpreted as instances of positive chemotropism. Moreover, there appeared to be an optimum concentration, beyond which such substances exerted a less and less attractive and at length a repellent effect. On the other hand, below the optimum, with gradually decreasing concentrations, the attractive effect also gradually decreased, ultimately to zero. Acids, alkalis, and some salts always produced a repellent effect, however, even in weak solutions.

Perhaps MIYOSHI's strongest point in support of his view was his statement that when no special chemical substances were offered, as, for example, when leaves were injected with pure water only, no turning resulted.

When we survey the investigations of FULTON (5) and CLARK (3), we find that, taken in combination, they completely contradict these results. CLARK injected leaves with various concentrations of copper and cobalt salts, etc., and found that in this case the germ tubes near the stomata curved toward and grew directly into them; but he obtained a similar result with leaves injected with pure water only. In experiments in which he used perforated mica plates, he found that the germ tubes near the perforation always "*grew toward the opening if it communicated with a layer in which no spores had been placed.*" Although he did not

follow out this line of work, he advanced the hypothesis that *Rhizopus* is "markedly chemotactic to some secretion of its own mycelium, and this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen."

The work of FULTON showed a decided advance in that, by introducing numerical methods of estimating the direction assumed by the hyphae, he placed on a numerical basis what had heretofore been more or less a matter of personal judgment. As PORODKO (**9**) has pointed out, in his work on chemotropism in roots, quantitative methods in this field must replace the qualitative ones hitherto used, if definite results are to be obtained. In startling contrast to MIYOSHI'S results, FULTON failed to find "the existence of any definite chemotropic sensibility to nutrient substances or other chemical compounds in solution." He believed that if positive chemotropism in the fungi exists, it is not as marked as turning caused by other stimuli. He states: "All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing, toward one destitute of hyphae, or in which the hyphae are less abundant. . . . . This may be regarded as a negative reaction to stimuli from chemical substances which owe their origin in some way to the growing fungus."

These results were so much at variance with MIYOSHI'S conclusions that they have been quoted with great reserve by such writers as BARNES (**4**), PRINGSHEIM (**10**), and JOST (**6**); and, on account of their conflicting nature, a restudy of the whole matter seemed imperative. The present work was undertaken, therefore, in the hope of deciding between the views of MIYOSHI on the one hand and those of FULTON and CLARK on the other.

Material and methods

Although the main part of the work was carried on chiefly with *Rhizopus nigricans* Ehrenb., preliminary tests were made also with *Botrytis cinerea* Pers. and a *Penicillium* which, after culture in the requisite media, answered most closely to *Penicillium* no. 24 Thom. Eventually the work was confined entirely to *Rhizopus* because its large spores and hyphae make the microscopic examination of the preparation easier; the comparatively short time necessary

for its growth reduces to a minimum the dangers both of contamination and of too extensive a diffusion of the substances tested; and the spores do not cling together in large clumps as in *Penicillium*.

For the experimental work, mica was selected on account of its impermeable nature. Small plates of this were cut from large sheets and perforated with a sharp needle. The area perforated measured 24×24 mm., while at two opposite sides of this square piece small winglike projections were left, in order to rest the preparation on supports. At first, the perforations were made 2 mm. apart, 121 holes thus being punched in each plate; but after experimental chemical tests demonstrated a comparatively rapid diffusion through such plates, the number was changed to 16, the holes then being spaced 6 mm. apart. After each experiment the mica plates were boiled, first in alkali, then in acid, and, after being rinsed and boiled in 2 or 3 changes of distilled water, they were dried finally in a hot air oven.

The spores were grown in thin layers or films of medium approximately 0.5 mm. thick, placed on these mica plates by a method to be described presently. For the medium, agar was used invariably, both because compared with gelatin it afforded less nourishment when used without addition to the substances to be tested, and because in the manipulation of the films, as explained later, it was found impracticable to use gelatin. A 1.5 per cent agar was used for the plain or "non-nutrient" medium; while for the nutrient a double strength solution of the required substance was mixed with an equal volume of 3 per cent agar to make a medium of desired strength containing 1.5 per cent agar.

The method finally adopted for making the films and sowing the spores was as follows. As already indicated, each preparation consisted of a perforated mica plate bearing a thin film of medium on each of its surfaces. The film on one side might contain the spores, while that on the other side would contain the chemical substances to be investigated; but various combinations were tried, as will be shown later.

For various reasons it was found desirable to make all films of approximately uniform area and thickness. An area approximately 24×24 mm., the size of the perforated area in the mica plates, was

marked out on a clean slide by means of a brush and a very small quantity of vaseline. Then on a warming table were placed a small beaker in which were water, a thermometer, and a small vial holding the agar for the films. When the agar, previously melted in hot water, had fallen to 40° or slightly below, the requisite number of drops of a suitable suspension of spores in distilled water was added. With a small pipette, marked to indicate the amount of agar solution it should contain for the making of a film, the correct quantity was drawn up, quickly dropped and spread evenly on the area marked out on the glass slide by the vaseline lines. In order to facilitate an even spreading of the agar, the slides were laid on a large glass plate fixed in an exactly horizontal position with the aid of screw supports and a spirit level. It was found that it was very essential to keep the agar agitated during the process of pipetting it from the vial; otherwise the spores would not be distributed evenly. Before the agar on the glass slide had had time to set completely, the mica plate was placed carefully upon it in such a way that the perforated area covered the film, care being taken not to press the mica down and thus force the agar up through the holes. Next, the same amount of agar of the required composition, from another vial, was dropped and spread on the surface of the mica plate up to the boundaries of the perforated area. Transverse grooves in the mica, cut for this purpose, in most cases confined the agar to the perforated area. In case no spores were needed for this upper film, several drops of distilled water had previously been added to the agar, which were equal in volume to that of the spore mixture added to the agar for the lower film. After the upper film was set, the whole preparation, consisting of the mica plate between the two films of agar, was carefully pushed off the slide with the help of a scalpel. It was on account of this last operation that it was found impracticable to use gelatin, since this adhered so closely to the slide that it was impossible to remove it without breaking.

The preparation was now rested in a horizontal position,[2] by means of the extensions at each end, on cork supports in a Petri

[2] In the earlier experiments, some of the preparations were suspended vertically by means of a thread passed through one of the holes in the mica plate. This vertical position was found to have no influence on the result.

dish, lined on all sides with filter paper moistened in distilled water. In this way the films were situated in an atmosphere practically saturated with water vapor, and each film was under exactly the same conditions as regards access of air. This last was a matter of great importance, since it was found in the earlier experiments that when the preparations were laid on a glass slide, the germination was always poorer in the film next to the glass slide, although normal germination occurred at or near the edges of the film.

To save labor, usually 2 or 3 preparations of the same kind were placed in a single Petri dish. In the preliminary experiments, these dishes had been placed directly in an incubator kept at a uniform temperature of 24–25° C. Even under these circumstances, however, it was realized that the atmospheric condition within the Petri dish might not be uniform throughout, for we know, of course, that Petri dishes so prepared eventually dry out. To obviate this, so far as possible, all the dishes (piled one above the other in a column, which rested on 2 or 3 empty dishes, or on an inverted stender dish) were stood in one of the halves of a larger Petri dish (9 in. diameter) which also was lined with several thicknesses of filter paper and contained a quantity of distilled water. The whole was then covered with a bell-jar, likewise lined throughout with filter paper, and resting in the larger Petri dish so that water was continually being drawn up by the filter paper. In this way the atmosphere within the small Petri dishes was kept as close as possible to a uniform saturation point throughout each dish.

The whole apparatus was now incubated at 24–25° C., and when the germ tubes had grown to the desired length, they were killed by dropping a little 4 per cent formalin on the preparations. They could then be kept for several days if necessary, or until it was convenient to examine them microscopically.[3] For examination under the microscope, the preparation was laid very carefully on a

[3] The nature of this entire operation, and the amount of time necessary for its completion, made perfect antiseptic precautions impracticable. All that could be done was to have the apparatus scrupulously clean and sterile. As a matter of fact, the short time requisite for the incubation of *Rhizopus*, in few cases more than 10 hours, would allow bacteria little time for development, and no contamination was ever observed in these preparations.

glass slide, and since only the low power was necessary, no cover glass was used, on account of the danger of shifting the film.

In working out the results, it was found essential to get an idea of the amount of growth which had taken place. This was done by estimating the average length of the hyphae in the preparation in question. To obtain this average, only those hyphae near the holes were considered, since in the regions midway between these holes the conditions of nourishment differed from those in the immediate proximity of the holes; especially was this true in the case of germ tubes in films made of plain agar, with a nourishing substance on the other side of the mica plate. A definite area was selected near each hole, and the lengths of all the hyphae in this area noted. In this way 3 or more holes in different parts of the preparation were considered, or until a total of 15 hyphae had been counted and averaged.

Since, as will be shown later, the number of viable spores,[4] as well as the number of hyphae present, was found to have a marked influence on the results, it was desirable for comparative results to have the spore numbers approximately equal. In order to get a suspension of spores which would produce the desired number of spores to each volume of film covered by a square millimeter of surface, the following method was used. Spore-bearing mycelium was allowed to stand in water for a few moments and was then strained through a piece of muslin, which had previously been freed of its starch by thorough boiling and washing. The spore suspension was then tested as to its concentration by the use of a micrometer eyepiece containing accurately ruled squares, and by diluting, or by centrifuging and pouring off some of the water, was brought to the desired strength.

METHOD OF ESTIMATING THE INTENSITY OF THE CHEMOTROPIC REACTION.—In work of this kind, the method of estimating the reaction is all-important, since on it depends the accuracy of the

[4] It was found that the number of viable spores present bears a definite relation to the percentage of germination. When the spores were excessively numerous no germination at all resulted. The cause of this non-germination was not worked out experimentally, but it is probably referable to the same agencies discussed later in this paper; that is, the excretion of toxic products by the spores, in this case in the pre-germination stages.

results. For this reason it is necessary to describe in some detail the method which was elaborated. First, for delimiting the region about the hole within which the hyphae were to be considered, the following rule was employed. Only those hyphae growing from spores which chanced to be located in a cylinder of medium situated over the hole were taken into consideration. This cylinder was delimited as nearly as possible by the eye, in such a way that the center of its base coincided with the center of the hole, its diameter being 3 times that of the hole. Although the holes varied considerably in diameter (8–45 μ), it was found that the average was about 16 μ, and that, by turning the fine adjustment of the microscope a suitable number of revolutions, a thickness of medium would be traversed approximately equal to one diameter of the hole. In all cases the hyphae coming from spores directly over the hole, that is, in an inner cylinder with the hole as its base, were neglected, owing to the difficulty in many cases of assigning a definite direction to them. Thus the region in which the germ tubes were considered was a cylindrical zone concentric with the hole, and equal in width and height to one diameter of the hole.

It is of course clear that the whole purpose of the adoption of this arbitrary region was to secure uniformity of conditions for collecting the data. For the most exact work, indeed, the portion of the medium chosen for examination should correspond to a hemisphere whose base coincides with the base of the above described cylinder, and thus has a radius equal to 1.5 times the diameter of the hole. However, it is practically impossible to define the outline of such a hemisphere with any accuracy by focusing, and so a cylinder of the dimensions described was chosen as the nearest practical approximation.

The next step was to determine as accurately as possible the direction taken by the hyphae. An imaginary straight line was projected from the center of the hole to the spore itself, in cases where the germ tube was fairly straight (fig. 1); if, however, the germ tube curved markedly, this method was modified slightly, as explained below. This line was supposed to make angles of 45° with two other imaginary lines intersecting one another at right angles at the spore. The 4 regions, of 90° each, thus marked out by

these last two lines, were then called *A*, *B*, *C*, and *D*, with orientations as shown in fig. 1. The hypha was then classed as *A*, *B*, *C*, or *D*, according to the quarter in which it grew.

If the hypha curved markedly, only its tip was considered, since the ultimate effect of the reaction was expressed in this portion. In this case, the point of intersection of the two lines at right angles was located at the tip, this having been connected to the

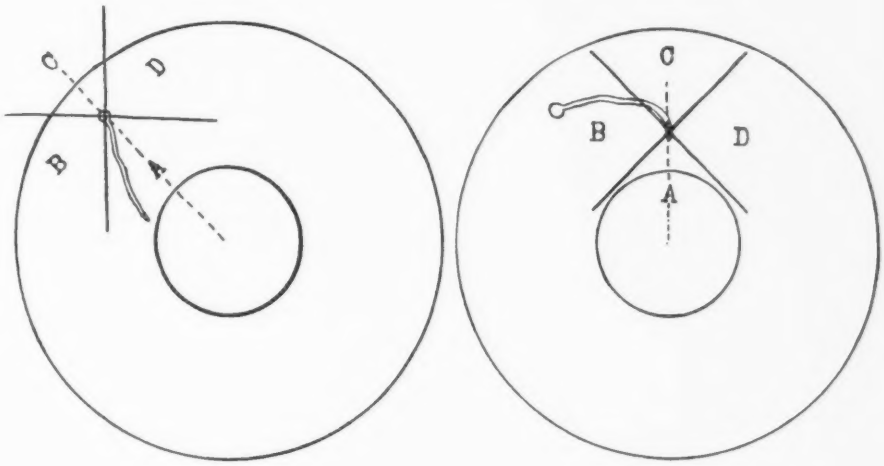

FIG. 1 FIG. 2

FIGS. 1 and 2.—Diagrams showing methods of locating directions of hyphae: fig. 1, where the hypha is fairly straight; fig. 2, where it curves markedly.

center of the hole by a straight line, as before. The hypha was then classified according to the quarter into which the tip pointed (fig. 2). In the few cases where the hyphae curved considerably up or down from their original plane, that is, above or below an optical section of the medium, their direction was taken as that which they would assume if projected on their original plane. Those growing vertically up or down in the medium were neglected.

The holes in the mica plates were examined in regular succession, one row after the other, in order that no subconscious selection could be made of those that appeared more favorable to the

results expected. The data for each hole were noted down separately, and the total was figured up when the preparation was finished. For the most part, for the reasons already stated, 16-holed plates were used, and usually 10 holes in each plate were examined.

The basis of the interpretation of the figures thus obtained lay in the assumption that the average number of hyphae in each of the 4 classes, considering a large number of holes, and supposing that no attractive or repellent forces existed, should, on the doctrine of averages, be approximately the same, that is, 25 per cent of the whole number; or, to put it in general terms, as many hyphae should point in one direction as another. That this assumption is legitimate is shown by the test count described later. Of the whole number, then, 25 per cent would constitute the normal number for each class, and any considerable deviation from this would indicate a reaction to a disturbing force.

Since the *B* and *D* regions are of equal volume and equally subjected to repellent or attractive forces, they may be left out of consideration except in so far as the number occurring in them goes to make up the total for each hole, and thus influences the final percentage of reaction. In order to determine this percentage, then, we are concerned first of all with the *A* and *C* classes. Let us assume that an attractive force exists, derived from a substance on the other side of the mica plate, which is causing the hyphae to a certain extent to grow toward the holes. Since the number of the *A* class will then be greater than the normal number, we must conclude that those in excess of one-fourth have been attracted into this class. If the attractive force is not a very strong one, as indicated by a number not greatly above the normal, we may be fairly certain that the increment has been derived from those which would normally point right or left, that is, those of the *B* and *D* class. The number of *A*'s in excess of the normal, however, does not give us a complete measure of the attraction, for in all probability some hyphae originally in the *C* class have turned into the *B* or *D* direction, and the remaining *C*'s will therefore be fewer than one-fourth. The difference between the remaining *C*'s and the normal number would then represent those which have turned from the *C* direction into *B* or *D*. By adding this number to those in excess

of the normal number in A we get the total number affected. In order to get the total percentage of reaction, we must divide this number by three-fourths of the total number of hyphae counted in the whole area, since we are dealing with only three-fourths of the hyphae, the one-fourth represented by the normal A's being unaffected, at least visibly.[5]

If we denote the normal number to be expected in each region by n (one-fourth of the whole), the number actually in each region by its corresponding letter, and the total number of hyphae in the whole area by t, the formula will then read as follows: $\frac{(A-n)+(n-C)}{\frac{3}{4}t}$; or, simplifying, $\frac{A-C}{\frac{3}{4}t}$ = percentage of hyphae reacting. In case a repellent force exists, raising the number of C's above the normal, the final percentage will then be a minus quantity, and it is so expressed in the accompanying tables.

One objection to this method lies in the possibility that some of the normal C's may have been attracted so strongly as to have turned from C, *through* the B or D areas, to A; these would then be counted twice over, for they would be included in the gain to A as well as in the loss from C. It is of course quite impossible to determine for a large number of holes how many hyphae have so acted. The objection, however, is not a serious one; for, as a matter of fact, when the number of B's and D's is fairly large, indicating a force not strong enough to draw them all into the A class, we know that the probabilities are much against the force attracting any from the C class into the A class.

However, when *more* than three-fourths of the total number point in the A direction, we can fairly assume that all which were originally B's and D's have now turned to the A direction; that is, of this 75 per cent, 25 per cent consist of those originally in A, and 50 per cent must have been derived from those nearest the A class, namely B and D; thus any number above 75 per cent must have come from those which were originally growing in the C

[5] Just what individuals among the normal A's have reacted to the stimulus we cannot of course judge, since by their original chance position they are all oriented toward the holes, but the proportion would correspond essentially to that for the remaining 75 per cent of the hyphae.

direction. In other words, chemotropic forces which would cause a turning of slightly more than 90° would bring all the *B*'s and *D*'s to *A;* but if the force were stronger than this, some of the *C* class would turn to the *A* direction. It is clear, therefore, that the number in excess of 75 per cent represents those which have turned from *C* to *A;* and that in this case those remaining in *B* and *D* must have been originally in the *C* class but have not turned strongly enough to become *A*'s.

Accordingly, whenever the total number of hyphae in *A* exceeds 75 per cent of the whole number, a correction must be applied; we must now subtract from our formula the number which have come over from *C* into *A*, to avoid counting them twice. We can ascertain, indirectly, how many of the *C*'s have so turned into *A* by subtracting the number left in *C* from the normal number, and by also subtracting from this result the sum of those left in *B* or *D;* for, as was previously shown, those now in *B* and *D* must also have been originally *C*'s. The remainder, representing the difference between all these and the normal number, will then denote the number which have turned from *C* through the *B* and *D* classes to *A*. In accordance with this, therefore, we must subtract from our first formula $[(n-C)-(B+D)]$. The whole formula would then read: $\frac{(A-n)+(n-C)-[(n-C)-(B+D)]}{\frac{3}{4}t}$; or, simplifying, $\frac{A-n+B+D}{\frac{3}{4}t}$ = percentage of hyphae reacting. As before, should the excess over 75 per cent be in the *C* class, the result of the calculation would be a minus quantity, and would indicate repulsion.

THE EXPERIMENTAL ERROR.—In order to test the accuracy of the method, 10 preparations were made with conditions as regards number and length of the hyphae, and composition of the medium, essentially the same in both upper and lower films. Under these circumstances, in accordance with our hypothesis, there should be no more turning in one direction than in another. Hyphae were examined in the prescribed manner for 10 holes in each preparation, making 100 holes for the entire operation, or, if we count both upper and lower films, in reality 200 lots of hyphae. The results, in the order in which the counts were made, are shown in table I.

It is evident from the results shown in table I that for small numbers, from 100 to 200, the experimental error may vary from 0 to 16 per cent; but by taking a large number, such as 774, the error is reduced to about 10 per cent. Also, apparently owing to the personal equation of the observer, the error is always minus.[6]

TABLE I

RESULTS OF TEST COUNTS TO DETERMINE ACCURACY OF METHOD

Preparation	Class				Total	Percentage of error
	A	*B*	*C*	*D*		
1	50	34	58	38	180	− 6
2	57	34	57	43	191	0
3	33	34	52	37	156	−16
4	24	28	39	36	127	−16
5	21	18	32	23	94	−16
					748	
	(Average error for 748 hyphae = −11 per cent)					
6	29	31	35	35	130	− 6
7	54	51	73	55	233	−11
8	41	29	49	20	139	− 8
9	25	28	36	33	122	−12
10	33	30	48	39	150	−13
					774	
	(Average error for 774 hyphae = −10 per cent)*					

* The same average error of 10–11 per cent was obtained when a recount was made after a few days' interval, so that the method can be trusted to give uniform results.

It is also noteworthy that the large error, up to 16 per cent, occurs only when the hyphae are not markedly turned in any one direction. In the results given later, where a marked turning occurs, the error would be much smaller.

FULTON'S METHOD.—In this connection, the method employed by FULTON (5) may be considered. It is stated as follows:

Hyphae within a radius of one opening diameter from the margin of each opening were considered, the hyphae within such an area were classed in the counts as those turning toward the openings, those turning away from the

[6] It is possible that the extra number in the *C* class may be due to some factor hitherto overlooked, which causes a slight repulsion from the holes on both sides of the plate, even when the conditions seem uniform in the two media.

openings, and those apparently indifferent. After an examination of the entire preparation in each case, those holes were selected for the counts which represented the average condition. In calculating the percentages from the counts the difference between those attracted and those repelled was made the dividend, and the total number within the observed area was made the divisor.

This method is open to at least 3 objections. In the first place, no definite method is given for distinguishing between the 3 classes of hyphae, that is, between the indifferent hyphae on the one hand, and those turning away from or toward the openings on the other hand. Without some such definite rule as that used in the method employed in the present paper, the matter of deciding their directions is likely to be influenced to a large degree by the personal equation, on account of the great variety of curves and angles which the hyphae assume. In the second place, apparently *all* the hyphae found in the area at the time of examination were included. It is clear that by such a method one must include some of the hyphae coming from spores located outside the special area; and if we are right in concluding that FULTON's classification of directions is similar to ours, it is evident that in this way a portion at least of the *A* class hyphae belonging to an outer zone would be counted, whereas the *B* and *D* classes would be mostly, and the *C* class hyphae of that zone would be entirely, neglected. Thus, even if there were no turning toward the holes at all, and the number of the *A* class in the prescribed zone were only the normal 25 per cent, by counting in some of the *A* class from the zone outside, the resulting figures would inevitably, though falsely, indicate the existence of a chemotropic reaction. Moreover, this error would be greater as the amount of real turning increased, and it would also increase with the growth in length of the hyphae, since eventually some of those from zones still more remote would reach into the prescribed zone. In the third place, although the area in the plane of the hole was clearly defined by FULTON, no statement was made as to the hyphae found in the parallel planes above the hole. If these were estimated in the same way as those in the plane of the hole, FULTON was really considering the hyphae in a cylinder of medium standing over the hole and reaching to the free surface of the film. If this were the case, he must have counted in hyphae

outside of his prescribed 1.5 diameters from the center of the hole, for in all probability his films were thicker than this.

THE RATE OF DIFFUSION THROUGH THE PERFORATED MICA PLATES.—In view of the researches of BROWN and ESCOMBE (2) on diffusion through perforated membranes, it was realized that it was essential to obtain definite data as to the rate of diffusion through the perforated mica plates. Experiments were set up in which the rates of diffusion of cane sugar through 121-holed plates and through 16-holed plates were compared for various periods of time. Without going into the details of the experiments, it may be stated that in either case, although diffusion did not proceed at such a rate as to reduce markedly the difference in concentration in the two films, yet diffusion through the 16-holed plate was naturally slower, and on this account plates so perforated were chosen for the work.

Experimental work

It is not our purpose to present a detailed account of all the experiments. In the following the most important results are outlined, using the data from representative tests. In all cases given, the results have been repeatedly verified. Altogether over 40,000 hyphae were examined and allocated to their 4 classes in the manner described above.

EXPERIMENTS WITH "STALED" SOLUTIONS.—Very early in the work, while various percentages of sugars were being tested for their power of inducing a positive chemotropism, it became quite evident that the hyphae would always turn toward the holes leading to the other agar layer, regardless of whether sugar was present in it or not, provided it contained no germinating spores. It was also significant that the more hyphae there were present in the one film, the stronger was the turning from it toward the film without spores. Furthermore, if the spores were sown in both layers, no turning resulted in either layer, or it was not nearly so pronounced. This was quite in line with the experience of FULTON and CLARK, and according to their hypothesis, previously stated, was probably due to a negative chemotropic reaction to some substance or substances excreted by the hyphae themselves. In accordance with

this theory we may thus conceive that the layer in which the hyphae were present would be permeated with their excretions, which in this paper have been termed the "staling substance or substances" (cf. BALLS 1, pp. 559, 576 ff.). Toward the region of the holes the concentration of this staling substance would be less and less, since here it is diffusing through to the other layer. This decreasing gradient of concentration would therefore act as a directive stimulus to the hyphae, causing them to grow toward the regions of less and less concentration, and thus through the holes.

In order to prove this hypothesis, the problem before us was to obtain, if possible, this staling substance free from mycelium, and determine whether, if placed in a sporeless layer, it would prevent the hyphae from growing into that layer. Although cane sugar and glucose were tried in order to obtain vigorous cultures of *Rhizopus* mycelium, and hence a strongly staled solution, the results were unsatisfactory. No medium tried gave anywhere near the vigorous growth that developed in turnip juice, prepared by pressing the juice from autoclaved white turnips.

Several experiments were performed, with unsatisfactory results, as regards a turning away from the medium containing the staled juice, the failure probably being due to the fact that the staling substances were not of the requisite strength. It was eventually found that the desired effect could be produced by allowing the juice to stale for from 3 to 4 weeks, that is, to grow the fungus in it for that length of time. For this purpose sterilized, conical flasks containing 10–15 cc. of sterile turnip juice were inoculated with *Rhizopus* and kept in an incubator at 25° C. In a few days a white weft of mycelium developed, and later sporangia appeared. By the end of 3 weeks the mycelium had taken on a brownish color, and growth had apparently ceased. The liquid remaining was now poured out of the flask. It gave an acid reaction to litmus and had a slightly sour odor of malt. Chemical tests showed the entire absence of oxalic acid or oxalates. The staled solution was now evaporated to one-half its volume, that is, to double strength, at laboratory temperature under reduced pressure. For making up the medium for the films, either one part of 6 per cent agar to 3 parts of the staled solution, or equal volumes of 3 per cent

agar and staled solution were used, in order to obtain a 1.5 per cent agar medium.

Table II gives the results of one series of experiments. In preparing the medium for this, care was taken not to heat the staled solution above 40° C.

TABLE II

NATURE OF EXPERIMENT { Mica plate → Staled turnip juice agar +non-viable spores* / Fresh turnip juice agar +viable spores

Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores in lower layer per sq. mm. of film surface	Direction of hyphae				Total number of hyphae counted	Percentage of reaction
				A	*B*	*C*	*D*		
2.....	7	67	28	17	37	77	21	152	−53
4.....	8.25	157	39	91	87	152	90	420	−19
2.....	10	331	29	45	48	91	48	232	−26

* In this case the staled solution was not centrifuged, and this accounts for the presence of spores in this layer. They were probably dead, however, and in any case did not germinate. Their presence may or may not have had its effect on the result, but it is extremely doubtful whether they influenced it to any marked degree. Similar results were obtained in series where the solution had been freed of its spores by centrifuging.

Ten holes in each preparation were counted. As would be expected, the turning away from the holes is most marked in the youngest stage, where the hyphae have not yet produced, by their own activity, a concentration of staling substance in any way comparable to that of the other film.

In preparing these films, equal volumes of the staled turnip juice and 3 per cent distilled water agar had been used. This process diluted the staled juice to one-half strength, or, in reality, to its original strength before evaporation. One would expect, under these conditions, to see an even greater repellent effect than the results show. It is very probable, however, and is also indicated by the following experiment, that the repellent substances are of an unstable or volatile character. Although, in order to avoid any chemical change in the substance, at no time in the making of the preparations was the staled juice agar heated above 40° C., it is

possible that even this degree of heat as well as the delay due to evaporation may have led to an alteration of the staling substances.

Table III shows the results of experiments to determine whether raising the staled juice agar to the boiling point altered its repellent effect (cf. figs. 3 and 4).

TABLE III

NATURE OF EXPERIMENT { Mica plate → Staled turnip juice agar / Fresh turnip juice agar + spores

Temperature	Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
Staled juice not heated over 40° C.	2.....	7.50	69	21	240	−26
	2.....	8.75	200	29	310	−28
Staled juice heated to 100° C.	2.....	7.00	57	31	168	+18
	2.....	8.50	143	23	218	+72

In this series the staled solution used was 3 weeks old, and, in order to dilute it as little as possible, one part of 6 per cent agar was added to 3 parts of the staled juice, a rather difficult operation in the case where there was no heating above 40° C., owing to the fact that at this temperature 6 per cent agar is very close to its gelatinization point.

The results show that the repellent substances of the staled solution are as a whole, or in part, unstable or volatile in character (figs. 3, 4). If they had been entirely destroyed, we should get results similar to those described later for *C* (p. 357), that is, 100 per cent of turning. It is possible, however, that the heating entirely destroyed the repellent substances, and that the smaller reaction as compared with the preparations cited is due to the fact that most of the substances in turnip juice which induce a positive chemotropism have been used up by the fungus which originally produced the staled solution.

In this connection the work of LUTZ (7) is of interest. In the investigation of the effect of used solutions on spore germination and fungous growth, he found that in many cases high temperature

(80–100°) destroyed the effect of these solutions, or at least temporarily altered it. Furthermore, he remarks, "Wenn sich zeigen lässt dass die uns interessierenden Stoffe durch Kochen zerstört werden, so liegt die Annahme nahe dass es sich bei ihnen vielleicht um fermentähnliche Körper handelt" (**7**, p. 106).

The theory advanced by CLARK and FULTON, therefore, that during their growth the hyphae produce some substance or sub-

FIG. 3.—Germ tubes of *Rhizopus nigricans* growing away from the hole; film on the other side of mica plate contains strong "staled" turnip juice; hyphae are growing in a medium composed of unstaled turnip juice agar; ×80.

stances to which they are negatively chemotropic, is fully proven by the present work, being supported by 3 lines of evidence: (1) the hyphae turn away from a film without spores if it contains their own staling substances; (2) the hyphae always show a marked turning from their own medium to a second one without hyphae, no matter what the constitution of either medium may be, unless, indeed, the second medium contains substances which exert a negative chemotropic stimulus; (3) when an approximately equal

amount of mycelium occurs in 2 films composed of the same medium, no turning results.

EXPERIMENTS WITH UNSTALED TURNIP JUICE.—After the existence of a negative chemotropism of this kind was proved, it became easier to search for a possible positive chemotropism. For, without going into detail, it was clear, from the fact that the hyphae are continually producing a staling substance, that the number of spores and the length of the hyphae must be considered when one is seeking to obtain evidence for positive chemotropism.

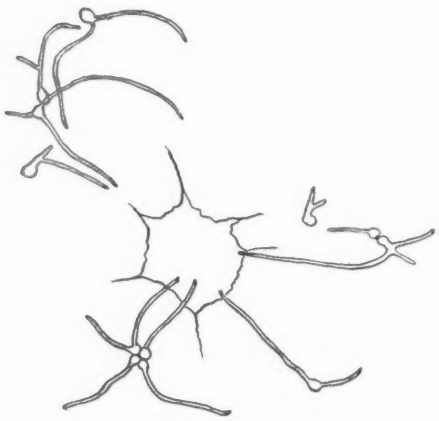

FIG. 4.—Germ tubes growing toward the holes; "staled" turnip juice agar has in this case previously been heated to 100° C.; otherwise conditions are the same as in fig. 3; ×80.

In the experiments with agar made with unstaled turnip juice, the first definite indication of a positive chemotropism (working, it is true, side by side with the negative chemotropism previously demonstrated) was obtained. The hyphae grew much more vigorously in the turnip juice medium than in the sugars. They did not in all cases grow faster; the chief difference consisted in the thickness of the germ tubes, which were twice or three times as thick as those grown in glucose or in cane sugar agar. Probably on account of this healthier development they reacted much better to chemotropic influences than when grown in the sugars. The agar was prepared by mixing equal volumes of turnip juice with 3 per cent distilled water agar, thus forming a medium containing 1.5 per cent agar. Combinations were tried as follows, the amount of reaction in each case being given below:

	A	*B*	*C*
Mica plate →	Plain agar / Turnip juice agar +spores	Turnip juice agar / Plain agar +spores	Turnip juice agar / Turnip juice agar +spores
Reaction →	+60–90 per cent	+100 per cent	+100 per cent

Since varying conditions of light, heat, and moisture had been eliminated, it is obvious that only chemotropic forces were at work here.

The turning toward the holes in preparations *B* and *C* was very marked, but in *A* it was not nearly so pronounced. The only difference between *B* and *C* lay in the distance from the hole at which the curvature became noticeable in the same period of time (8.5 hours). In *C*, the reaction was apparent at a distance of 3–4 diameters of the hole, counting from its margin; but in *B*, the turning could be observed as far as 10 diameters from the hole. In *B* and *C* the turning in all cases was 100 per cent, while in *A* it varied from 60 to 90 per cent.

In *C*, since the turnip juice was everywhere of practically the same concentration, the only force exerted must be due to the staling substances produced by the hyphae, that is, a negative chemotropic force. In *B*, however, where we have the most marked turning of all, we can fairly assert that the turnip juice in the sporeless layer is the cause of the additional stimulus, and is, therefore, a *positive* chemotropic force. This reasoning was corroborated by the condition in *A*, where in the lower film the positive chemotropic force, due to the turnip juice, would be working *against* the negative chemotropic force due to the staling substance, with the resulting decrease in the amount of turning.

In these experiments the hyphae had been allowed to grow to a considerable length, none being under 300 μ, since it was reasoned that with long hyphae the negative chemotropic stimulus would be much greater than with short ones. On the other hand, the positive chemotropic force should be exerted just as strongly on short hyphae as on long ones. In view of this, younger stages were tried, with results such as are set forth in table IV.

The combinations *A* and *B* correspond to those given previously, but *C* and *D* are new. *D* represents the control, for here conditions were made as much alike as possible in both upper and lower films. The turnip juice being everywhere practically the same in amount, no positive chemotropic force can be acting. If the number and length of the hyphae were also equal in both films, the turning on both sides should be no more pronounced in one direction than in

another. It will be seen, however, that in every case the figures fall within the recognized experimental error.

TABLE IV

RESULTS IN RHIZOPUS NIGRICANS WITH TURNIP JUICE AND PLAIN AGAR; YOUNG STAGES OF GROWTH

	Nature of films and location of spores	Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
A	Upper film: plain agar	2	6.75	40	37	108	+ 2*
	Lower film: turnip juice agar+spores	2	7.25	66	58	168	+18
		2	8.00	111	56	192	+43
B	Upper film: turnip juice agar	2	6.75	75	53	189	+94
	Lower film: plain agar+spores	2	7.25	136	49	206	+99
		2	8.00	142	46	198	+94
C	Upper film: turnip juice agar+spores	2	6.75	above 60	20	179	−13
				below 51	15	118	+17
	Lower film: plain agar+spores	2	7.25	above 80	23	158	− 8
				below 58	23	155	+33
		2	8.00	above 175	23	192	−30
				below 148	33	223	+46
D	Upper film: turnip juice agar+spores	4	7.00	above 60	21	272	− 8
				below 74	27	292	+0.5
	Lower film: turnip juice agar+spores	4	7.75	above 80	23	286	−6.5
				below 101	22	378	−2.0

* + and − signs indicate turning toward or away from the holes, that is, positive and negative reactions respectively.

The most marked turning is shown in *B*, where the hyphae while still very short (75 μ) show 94 per cent of reaction. Here we have both chemotropic forces exerting a stimulus in the same direction. A most interesting contrast to this is shown in *A*, where the turning is toward a plain agar film without spores. The turning is much less marked than in *B*, not in any case even half as great. The hyphae, indeed, are shorter, but this is balanced by the fact that on the whole the spore number, and consequently the number of hyphae, is greater. The result here, therefore, corresponds with the case of *A* among the older preparations described above, and the same observations apply here. Also, as would be expected in

the young stage, the effect of the negative chemotropic force is very slight where the hyphae are only 40 μ long. Later, when more staling substance has developed with the growth of the hyphae, the percentage of turning toward the film free from staling substance is much increased; we have seen in the older preparations that when the hyphae attain a considerable length the turning may become nearly 100 per cent.

A consideration of *C* bears out our interpretation of *A* and *B*, for since both films here contain spores, the amount of staling substance is more or less equal throughout the preparation, and the force due to a negative chemotropic stimulus, therefore, is practically eliminated. Any reaction which occurs should be a positive chemotropic one, and, as the tables show, there is a considerable turning from the plain agar to the turnip juice agar. That the extent of this increases with the age of the preparation is not easily explained, though it is no doubt partly due to the fact that some of the hyphae naturally react more slowly than others.[7] On the other hand, the hyphae in the turnip juice agar show an evident repulsion from the plain agar. This repulsion also increases with the age of the preparation, although not so markedly. It may likewise be accounted for by a slower response of some of the hyphae, as well as by the fact that in this film the concentration of the turnip juice decreases, through diffusion, at first only in the immediate vicinity of the holes; later the decreasing concentration extends farther out from the holes and affects more hyphae.

EXPERIMENTS WITH CANE SUGAR.—Strengths of 2.5, 5, and 10 per cent cane sugar were tried, the cane sugar used being the ordinary commercial lump sugar. The following combinations were arranged in the experimentation with each percentage of sugar.

	A	*B*	*C*
Mica plate →	Plain agar / Agar+sugar +spores	Agar+sugar / Plain agar +spores	Agar+sugar +spores / Plain agar +spores

In 2.5 per cent cane sugar agar, the hyphae appeared very ill nourished, being slender and of slow growth. It seems reasonable

[7] It may perhaps be caused also by the continual diffusion of the turnip juice farther and farther from the holes into the plain agar, thus acting on more and more hyphae.

to suppose that on this account they reacted weakly to chemotropic stimuli. As would be expected, the greatest turning was shown in *B*, where, when the hyphae were fairly long (200 μ) and sufficiently abundant, the percentage of reaction sometimes reached 40–50 per cent. That this turning was mainly due to a negative chemotropic reaction is apparent from what follows.

The experiments with 5 per cent cane sugar gave the best results, since this proportion seemed to supply the hyphae with a better amount of food, and also exerted a stronger positive chemotropic stimulus. Table V gives the results in condensed form.

TABLE V

RESULTS WITH 5 PER CENT CANE SUGAR

Combinations (as given in diagram above)	Number of preparations examined	Average length of hyphae in μ	Number of germ tubes per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
A	3	116	17	162	+ 6
	4	133	25	282	+ 9
	4	155	19	195	+12
	3	189	23	185	+32
B	3	115	10	99	− 2
	3	139	14	150	+37
	3	162	12	184	+35
	4	197	14	229	+55
C	11	above 204	above 18	above 253	above −19
		below 191	below 5	below 280	below −10

Since it was clear from the experiments with turnip juice that the strength of the negative chemotropic stimulus was directly related to the length of the hyphae, the attempt was made to compare preparations containing hyphae of approximately equal length. Thus, the 4 lots of preparations in *A* and *B* roughly correspond. Unfortunately, the number of germ tubes averages much less in *B*, for many of the spores did not germinate, but if it were greater, assuming that it would be accompanied by an increased negative chemotropic stimulus, the final percentages in *B* would even be greater than they now are.

It is evident from the percentages that the hyphae in every stage of growth in *B* except the youngest are subjected to much

greater chemotropic stimuli than is the case in *A*. This agrees well with the experiments with turnip juice; in *A* we have the negative chemotropic stimulus working against the positive chemotropic stimulus, while in *B* we have both these forces working together.

The fact that no positive curvature appears in the early stage of *B* may be accounted for by the weak growth of the hyphae in non-nutrient agar. They thus do not react to the sugar diffusing from the upper film, nor have they yet produced sufficient staling substances to cause them to turn to the holes leading to the upper layer. But with the increasing diffusion of sugar they become in time better nourished, develop more of the staling substances, and react to this negative chemotropic stimulus as well as to the positive stimulus exerted by the sugar.

In *C*, although the average number of germ tubes per sq. mm. in the lower layer is given in the table as 5, a result of the poor germination in the plain agar, in this case among the 11 preparations holes were selected which had an approximately equal number of hyphae about them in both films. In order to have germ tubes of fairly equal length in both lower and upper films, the lower films were given a 4 hours' start before the upper films were added. The results, however, are not in line with what we have seen for the same experiment with turnip juice (*C* of table IV), for we would expect to find a positive reaction in the lower layer; but, as already observed, the spores germinate poorly and the hyphae grow slowly in the plain agar. The majority of them germinate only around the holes, where the sugar is diffusing through. With this sugar diffuse also staling substances from the more vigorously growing hyphae above, thus counteracting any stimulus which would otherwise be exerted on the hyphae of the lower layer by their own staling substance. The positive result in the similar case where turnip juice is used is probably due to the greater chemotropic activity of the turnip juice; and possibly also to a more rapid specific diffusion of the active substance of turnip juice as compared with cane sugar, as well as to the more vigorous growth of the lower layer hyphae which are nourished by the diffusing turnip juice.

With 10 per cent sugar, the results confirm the preceding.

EXPERIMENTS WITH GLUCOSE.—Glucose in strengths of 2.5, 5, and 10 per cent was also tried in the same way as described for cane sugar. The hyphae grew more vigorously in the glucose and reacted better. The results, in general, agree with those given for cane sugar.

MIYOSHI'S TEST BY DIRECT APPLICATION OF THE SUBSTANCES.—Among other tests for chemotropism, MIYOSHI (**8**) sowed spores in a film of 5 per cent gelatin on a glass slide, and when the germ tubes were still quite short, he placed a small amount of glucose at a given point in the gelatin. Diffusion commenced immediately, of course, and spread radially through the gelatin. With *Rhizopus*, he found that although some of the hyphae showed only more copious branching and an increase in thickness, others curved decidedly toward the center of diffusion. With *Penicillium*, however, no effect could be observed. On experimenting with other substances, he concluded that whenever no positive results were obtained, the failure was due to too rapid diffusion of the chemical substances.

Since this method was simple, and appeared practical, it was tried out extensively by the writer, using agar as well as gelatin films, and adding in some cases a small bit of solid glucose, in others, of cane sugar. Small pieces of turnip juice agar were tried also, these being fitted into corresponding cavities in the films. Neither *Rhizopus*, *Penicillium*, nor *Botrytis* gave any positive result, beyond the more copious branching and increased hyphal thickness mentioned by MIYOSHI. Even in the case of the turnip juice agar, no turning was evident on inspection with the microscope. Possibly, as MIYOSHI suggests, too rapid diffusion is the cause of the failure here. In any case, the mica plate method is far more definite and accurate.

Discussion

THE STALING SUBSTANCES.—From the foregoing it is clear that changes take place in a medium in which the fungus has grown, so that the medium then acts in a negatively chemotropic way toward the fungus. Whether these changes are due to the excretion of

katabolic products by the hyphae, or whether they are the result of chemical changes which these excreted products induce in the medium itself, it is not possible to state with certainty. In any case, vital processes of the fungus are primarily responsible.

Of these two possibilities, however, it is much more probable that the fungal excretions are themselves directly the cause of the negative chemotropic action, chiefly on account of the fact that *all* the media worked with produce a negative chemotropic reaction after the fungus has grown in them for a time. It is unlikely that with these various kinds of media the same repellent substances would invariably be formed by chemical action of the fungal excretions. In support of this view, it is also noteworthy that with *plain agar* the same repellent effect was evidenced as with other media.

That the staling substances are either of a volatile or of a thermolabile nature has already been conclusively demonstrated. BALLS (1) has also shown this, and has ascertained that they exert an inhibitory effect on growth.

RELATIVE VALUES OF POSITIVE AND NEGATIVE CHEMOTROPIC FORCES.—The great difference of various germ tubes in their capacity for reaction was very noticeable in the examination of the preparations. Thus, with two germ tubes at an early stage of growth, and at an equal distance from the hole, one would react markedly, while the other remained indifferent. At a later stage, however, when we may assume that the stimulus was greater, all of the germ tubes within the prescribed region about the hole might react.

Our knowledge of the relation between individual variation in sensitiveness of the germ tubes and the intensity of the acting stimulus is of course very vague,[8] but it seems safe to assume that a larger percentage of turning means a stronger stimulus. Also the rate of diffusion of the stimulating substances would, within limits, affect the number of germ tubes reacting in a given volume

[8] It is probable that the variation curve of sensitiveness of the germ tubes is of the well known "normal" type, so that the percentage reacting is not directly proportional to the strength of the stimulus. TRÖNDLE (12) has shown that for geotropic sensitiveness the curve is of this type.

of material around the hole; and again, the time during which the stimulus had been acting (since a longer period gives greater opportunities for growth) would also affect the result. Bearing all this in mind, we nevertheless seem justified in assuming that under the conditions of any given experiment the percentage of turning gives some measure, although only a very approximate one, of the intensity of the stimulus or stimuli acting.

By an inspection of the results, especially as shown in tables IV and V, we can thus arrive at some idea of the relative intensity of the negative as compared with the positive chemotropic stimulus. For if we denote by n the percentage effect due to the negatively acting staling substances, and by p the percentage effect produced by the positive stimulus, we have in *A* of table IV, using the percentage of reaction in the oldest preparations, $n-p=43$; whereas in *B* we have $n+p=94$, taking the two oldest preparations here also. Since the hyphae in these two corresponding cases are of approximately equal length, and have therefore probably produced an essentially equal amount of staling substance, a comparison is legitimate. By eliminating the p's, we have $2n=137$, or $n=68.5$; p will then be 25.5. In other words, in the special condition of this experiment, the positive chemotropic stimulus exerted by the turnip juice has an effect of a little more than one-third that of the negative chemotropic stimulus. In a general way these figures are corroborated by the results given for *C*.

In the same way, applying this method to the results given in table V, we may arrange the relative values of the stimuli as shown in table VI.

With the exception of the youngest hyphae, where diffusion of the chemotropic substances has hardly begun, or at least the hyphae have not yet had time to react to it, the figures are surprisingly regular, the percentage of the reaction due to the positive chemotropic stimulus remaining fairly constant, as would be expected. On the other hand, the negative chemotropic stimulus constantly increases with the growth of the hyphae and the excretion of more and more of the negatively chemotropic substances.

In *C* of table V we should expect to find a confirmation of these deductions, as was shown by *C* of the turnip juice experiments

(table IV), but we have already seen why there is a discrepancy in the results for the lower film. On the other hand, the −19 per cent in the upper film is not greatly in excess of the amount representing the positive stimulus in *A* and *B*, for since the sugar in this case is in the upper film, the stimulus resulting from this would attract the hyphae away from the holes, and thus cause a negative figure.

TABLE VI

RELATIVE VALUES FOR POSITIVE AND NEGATIVE CHEMOTROPIC STIMULI IN CANE SUGAR EXPERIMENTS

Average length of hyphae	Nature of preparation	Percentage of total reaction	Percentage of reaction due to positive stimulus	Percentage of reaction due to negative stimulus
100–125	A	(+ 6*)	(− 4.0)	(+ 2.0)
	B	(− 2)		
125–150	A	+ 9	+14.0	+23.0
	B	+37		
150–175	A	+12	+11.5	+23.5
	B	+35		
175–200	A	+32	+11.5	+43.5
	B	+55		
Very long†	A	+49	+11.0	+60.0
	B	+71		

* The results in brackets all fall within the experimental error.

† These figures are taken from another series of experiments, not included in table V.

COMPARISON OF THE CHEMOTROPIC STIMULUS CAUSED BY CANE SUGAR AND BY TURNIP JUICE.—The cane sugar medium of 5 per cent strength, which seemed to be the proportion most favorable for growth, exerts, therefore, a comparatively small positive chemotropic force. Since it has been shown that when the turning is not very marked, the counting tends to be about 10 per cent too low, these numbers should probably be increased by about that percentage. But even if this be done, in comparison with it the stronger positive chemotropic force manifested by turnip juice is extremely striking and suggestive in relation to problems of parasitism, since it may indicate that plant juices in general evoke a fairly high positive chemotropic response.

Further work with plant juices is much to be desired. These juices of course contain a large variety of substances, the combined effect of which is evidently (at least in turnip juice) much more powerful than that of cane sugar. This is perhaps one of the reasons why FULTON, working with only simple substances like the sugars and various salts, was unable to demonstrate the existence of the very weak positive chemotropism which these substances may cause. The fact should also be emphasized that the hyphae grew much more vigorously in turnip juice than in the sugars, and probably on that account reacted better as well as much earlier. It is reasonable to associate a stronger reaction with a healthier growth.

THE GRADIENT OF DIFFUSION IN ITS RELATION TO CHEMOTROPISM.—MIYOSHI (**8**) claimed that when hyphae are placed between two concentrations of a substance, the concentrations being kept constant, no chemotropic reaction occurred unless a definite ratio existed between the two concentrations. Thus a fungus between 0.1 and 0.3 per cent of a sugar solution would not react; but with 0.1 and 1.0 per cent, a positive curvature occurred toward the 1 per cent. Similarly a 0.5 per cent solution must have a 5 per cent solution as an antithesis, if a reaction is to ensue. In other words, the conditions for a sufficient stimulus to produce a turning are in accordance with Weber's law. In our own experiments, in the cases of the hyphae turning toward the holes, it is difficult to conceive how this law can apply. The hyphae are so slender that the difference in concentration of the diffusing substance on opposite sides of a hypha, assuming that the latter is growing tangentially to the hole, could hardly be in the proportion of 10 to 1, given by MIYOSHI as requisite for a reaction. JOST (**6**) has voiced a similar criticism. As a matter of fact, it is quite evident from our work that the hyphae must react to vastly smaller differences in concentration than this. We must admit, however, that the external factors which bring about chemotropic reaction are still somewhat obscure. Possibly there is a reaction to the direction of the diffusion current.

OSMOTROPISM.—It is possible that osmotropism, as suggested by PORODKO (**9**), may play some small part in the reactions, but it cannot be an important factor, for if it were, we should get similar

results with solutions of equal osmotic pressure. Now, as regards the osmotic pressure of turnip juice, W. BROWN, working at this college, has determined that it is equal to that of a 14 per cent solution of cane sugar. It will be recalled that in the preparation of the turnip juice agar the turnip juice was diluted to half-strength, and its osmotic pressure must therefore be equal to about a 7 per cent solution of cane sugar. But our experiments show that even a 10 per cent solution of cane sugar is far less effective for causing a reaction than the turnip juice. It is clear, therefore, that osmotropism cannot be of great importance.

THE DISTRIBUTION OF A FUNGUS IN ITS HOST.—While the results given in this paper apply only to *Rhizopus nigricans*, there is abundant evidence from similar work carried on with *Penicillium* and *Botrytis* that the same conditions exist there also. It is quite likely that similar chemotropic reactions obtain in the majority of the fungi. If this be the case, it is possible that the distribution of a fungus in its host may depend mainly, not on a positive chemotropic reaction, but on the dominant negative chemotropic stimulus of its own staling products.

Summary

The following results apply to *Rhizopus nigricans* Ehrenb. in particular, but experimental evidence is at hand that the general principles involved apply also to *Botrytis cinerea* Pers. and *Penicillium* no. 24 Thom. Most of the data have been derived from work with two layers of medium separated by a perforated mica plate.

1. The fungus shows a marked negative chemotropic reaction to a medium in which it has been growing for some time.

2. The hypothesis brought forward by CLARK and FULTON, that this negative chemotropism is a reaction of the fungus toward its "staling substances," is conclusively substantiated by the following evidence: (*a*) the hyphae in one layer of medium turn away from another layer without spores if it contains their own staling products; (*b*) the hyphae always show a marked turning from the medium in which they are growing to any medium which is free of hyphae, no matter what the composition of that medium may be, unless the second medium contains the substances which exert a negative chemotropic stimulus; (*c*) when approximately

equal amounts of mycelium occur in two similar layers of medium, no turning of the hyphae from the one to the other results.

3. These staling substances are formed as a result of the vital activities of the fungus itself. They consist probably of excreted products of metabolism.

4. The staling substances appear to be either thermolabile or volatile, for boiling a solution containing them reduces markedly their negative chemotropic influence.

5. Positive chemotropism toward the substances tested (turnip juice, cane sugar, and glucose) also exists; but under ordinary conditions of growth this positive chemotropism is very much weaker than the negative chemotropism previously mentioned.

6. Turnip juice exerts a much stronger positive chemotropic stimulus than the other simple chemical substances tested, much stronger than, for example, 5 per cent cane sugar. This suggests the possibility that plant juices in general evoke a stronger positive chemotropic response than the simple chemical substances heretofore experimented with. The nature of the attractive substance or substances in turnip juice has not yet been determined.

7. It is impossible in the present stage of our knowledge to compare accurately the strength of two chemotropic stimuli; but, using the number of hyphae turning as a test of the relative action of the two stimuli, we certainly may conclude that in comparison with the stimulus exerted by negative chemotropism, that due to positive chemotropism is very much less. For example, in a given preparation with two layers of media separated by a perforated mica plate, and with the hyphae at a certain stage of growth, when 90–100 per cent of them are turning toward the holes from plain agar to turnip juice agar, the stimulus due to the positive chemotropic effect of the turnip juice has, very approximately, one-third of the effect of the stimulus due to the negative chemotropism from the staling products of the fungus itself. In other words, positive chemotropism is responsible at this stage and under these conditions for about one-quarter of the whole reaction. With the sugars the part played by positive chemotropism is very much less and is *easily overlooked*.

8. In contrast to the increase in strength of the negative chemotropic stimulus with the age of the preparation, the

attraction resulting from positive chemotropism remains fairly constant.

9. If the preceding conditions are true of fungi in general, it is probable that the distribution of a fungus in its host is influenced mainly, not by positive chemotropism, but by the dominant negative chemotropism due to its own staling products.

10. The part played by osmotropism in these reactions must, if any, be a very small one.

In conclusion, the writer desires to express his great indebtedness to the invaluable suggestions and hearty cooperation of Professor V. H. BLACKMAN, at whose instance this work was undertaken. The main part of the investigation was carried on under Professor BLACKMAN'S direction at the laboratory of Plant Physiology and Pathology, Imperial College of Science and Technology, London, but the work was finished at the Osborn Botanical Laboratory of Yale University.

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LITERATURE CITED

1. BALLS, W. L., Temperature and growth. Ann. Botany **22**:558–591. 1908.
2. BROWN, H. T., and ESCOMBE, F., Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Roy. Soc. London B **193**:223. 1900.
3. CLARK, J. F., On the toxic properties of some copper compounds with special reference to Bordeaux mixture. BOT. GAZ. **33**:26–48. 1902.
4. COULTER, J. M., BARNES, C. R., and COWLES, H. C., A textbook of botany. Vol. I. Chicago. 1910.
5. FULTON, H. R., Chemotropism of fungi. BOT. GAZ. **41**:81–108. 1908.
6. JOST, L., Vorlesungen über Pflanzenphysiologie. Jena. 1913.
7. LUTZ, O., Über den Einfluss gebrauchter Nährlösungen auf Keimung und Entwicklung einiger Schimmelpilze. Ann. Mycol. **7**:91–133. 1909.
8. MIYOSHI, M., Über Chemotropismus der Pilze. Bot. Zeit. **52**:1–28. 1894.
9. PORODKO, T., Über den Chemotropismus der Pflanzenwurzeln. Jahrb. Wiss. Bot. **49**:307–388. 1911.
10. PRINGSHEIM, E. G., Die Reizbewegungen der Pflanzen. Berlin. 1912.
11. THOM, C., Cultural studies of species of *Penicillium*. U.S. Dept. Agric. Bur. An. Ind. Bull. 118. 1910.
12. TRÖNDLE, A., Untersuchungen über die geotropische Reaktionszeit und die Anwendung variationsstatistischer Methoden in der Reizphysiologie. Neue Berichte Schweiz. Naturf. Gesells. pp. 84. 1915.

STERILITY AS THE RESULT OF HYBRIDIZATION AND THE CONDITION OF POLLEN IN RUBUS[1]

CARL SHERMAN HOAR

(WITH PLATES X–XII)

One of the first well authenticated records of the possibility of crossing species dates from 1694. In that year CAMERARIUS (1) in a letter to VALENTINE mentions the effect of crossing the hemp (*Cannabis*) with the hop (*Humulus*). However, it was not until 1760 that KÖLREUTER (2) made the first plant hybrid which was to be of use to science. He crossed *Nicotiana paniculata* ♂ and *Nicotiana rustica* ♀. In stating his conclusions he recognizes the relative fertility of hybrids from crossed varieties as compared with the relative sterility of hybrids made between distinct species.

As a result of KÖLREUTER's research much interest was aroused among plant breeders as to the rôle of hybridization and as to the conditions which lead to sterility. Such men as KNIGHT (3) and HERBERT (4) will, perhaps, illustrate the diversity of opinion which sprang up. KNIGHT, on the one hand, held that hybrids from parents of distinct species were sterile, while, on the other hand, the varietal crosses were fertile (an opinion quite like that of KÖLREUTER). HERBERT, on the contrary, found that hybrids between different species are frequently fertile, a fact which he interpreted as signifying that the parents had branched from the same main stock. FOCKE (5) also has pointed out the fact that no peculiarity of hybrids has attracted so much attention as the lessening of the power of reproduction, and that crosses between more distantly related plants are more sterile even up to entire sterility. Other views have been expressed since, some favoring one side and others being equally sure that the contrary is true. However, though this diversity of opinion is prevalent even to the present time, nevertheless it seems clear that sterility is a common characteristic of hybrids.

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

Sterility may be brought about in several different ways, both physiologically and morphologically. My purpose is not to enter into the subject of physiological sterility, which is of much more interest economically than it is from the evolutionary standpoint; and, accordingly, I shall confine my attention entirely to the morphological side.

One of the most obvious types of morphological sterility is indicated by the sexual organs of the flower. Often as the result of hybridization the anthers and pistils are abnormal, and still more often, even when these are normal, we find a large percentage of sterile ovules present. The pollen shows this type of sterility especially well. I have records as far back as 1832 in which morphologically sterile pollen has been recognized as a criterion for hybridization. In that year DUTROCHET (6) wrote a letter to the Academy in which he attributed the sterility of hybrids in part to the imperfections of pollen. He refers to the flower of a hybrid cherry tree, resulting from the union of *Prunus cerasus* and *P. avium*, whose "stamina" formed a compact mass in which the pollen did not divide into pollinic or fertile dust as in the case of fruitful trees. This appears to be an extreme case, and yet it may rightfully be classed as a type of morphological sterility. GÄRTNER (7), in his well known essay, also refers to pollen sterility as a criterion for hybridization. Again, according to DUCHARTE (8), NAUDIN observed that the degree of fertility of hybrids was *en rapport* with the number of normal pollen grains formed in the hybrid.

Since the discovery of MENDEL'S work (9) a great impetus has been given to plant breeding, and we find many scientists entering into the problems opened up thereby. Most of the work done has been in regard to the inheritance of factors and to the working out of Mendelian laws of heredity. However, several observers have given their attention to the question of the pollen condition. ROSENBERG (10) discovered that in an apparently sterile hybrid sundew (*Drosera longifolia obovata*) the young pollen grains mostly lost their contents, while the embryo sacs usually did not develop beyond the binucleate stage. DETLEFSEN (11), working upon the animal side, has shown that a partial abortion of gametes occurs in the male progeny of a *Cavia* species cross.

Other interesting facts have been brought out from the cytological standpoint. Several investigators have worked at pollen sterility from this side, and though it is not my purpose to go into any prolonged discussion of the matter, yet it may be well to give a brief account of what has been done. JUEL (12), working with a hybrid known as *Syringa chinensis*, noticed that all the pollen was abortive and that abortion did not take place until after the formation of tetrads. TISCHLER (13), through his research upon the same hybrid, and also upon hybrids of *Ribes*, found that pollen abortion seemed to take place usually after the formation of tetrads. He also made a study of hybrids from a *Bryonia* species cross made by CORRENS, and found that the tetrads were formed but that the pollen generally aborted, as did also the embryo sacs. He ascribes the frequent sterility of mutants, accompanied by irregularities similar to those of hybrids during reduction, to the disturbance of the idioplasm. GEERTS (14), in working out the cytology of *Oenothera Lamarckiana* of DEVRIES, determined that one-half of the pollen from each tetrad aborted, and also that one-half of the embryo sacs did not develop. BELLING (15) speaks of this behavior as being unique, since, as he states, "these Oenotheras cannot be considered hybrids." This statement is worthy of note when taken into consideration with views recently expressed, not only about *Oenothera* as a genus, but concerning the Onagraceae as a whole. OSAWA (16) has recently shown that in the case of the Unshu (Satsuma) orange, although this is not known to be a hybrid, yet nearly all the pollen aborts and the abortion takes place only after the formation of tetrads.

As can readily be seen, all these writers emphasize the fact that the abortion of pollen seems generally to take place only after the formation of tetrads. DORSEY (17) not only points out that this seems to be the case with the genus *Vitis*, but he also distinguishes two kinds of abortion. To quote directly:

> In the formation of sterile and fertile pollen of the grape the heterotypic and homotypic divisions and the division of the microspore nucleus take place normally. Sterile pollen in the grape results from degeneration processes in the generative nucleus, or arrested development previous to mitosis in the microspore nucleus. When degeneration begins early after the division of the microspore nucleus, both the generative and vegetative nuclei may remain normal.

Before leaving the general question in regard to the formation of sterile pollen, I should like to call attention to another phase upon which no great amount of work has yet been done. This is in regard to Mendelian research. BRAINERD (**30**), in one of his articles concerning hybrids of *Viola*, describes violets which he has cultivated and which he knows to be hybrids from their external characters. BATESON (**22**) makes this statement: "Such an experiment raises the hope that sterility consequent upon crossing, the most obscure of all genetic phenomena, may become one of the possibilities of Mendelian research." JESENKO (**19**), using a cross between wheat and rye, was able to obtain one hybrid plant with pollen mostly shrunken and sterile. After several artificial crosses he was able to obtain a second generation hybrid which was apparently quite fertile. However, when he repeated the experiment on a larger scale, he obtained several first generation hybrids, but could obtain none of the second generation except through back-crosses. He now thinks that the first second generation plant which he obtained was of such a nature. SUTTON (**20**) also has done work in crossing varieties and species of cultivated peas, together with a wild pea of Palestine (*Pisum humile*), and his results have been confirmed by BATESON (**22**). Although he found the first generation hybrid to be either entirely or partially sterile, yet when he obtained second generation plants, he found them to be fertile. This may, perhaps, be explained by the fact that he used plants close in relationship and hence more compatible.

Within a few months a study of pollen sterility has been made by BELLING (**15**) in which he has attempted to show that certain ratios prevail. His work has been carried on in Florida, chiefly with 3 species of *Stizolobium*. The first, the Florida velvet bean (*Stizolobium deeringianum* Bort.), he states is of unknown origin, but is very commonly known as "a large ornamental tropical climber." The second, the Lyon bean (*Stizolobium niveum* Roxburgh), is commonly grown in the Philippines and elsewhere in South Asia. The Yokohama bean (*Stizolobium hassjoo* Piper and Tracy), which he takes for the third, is common upon the island of Japan. He has worked more especially with these, but some work, though only with the first generation hybrid, has also been

done by him with the China bean (*Stizolobium nivcum* var.?), commonly considered as a variety of the Lyon bean. Perhaps it is not well to go into more detail, but rather to allow the reader the privilege of consulting the article for fuller details. His conclusion is that, generally speaking, 50 per cent of the pollen of the F_1 generation loses its contents and becomes sterile. A similar degree of sterility seems also to hold for the embryo sacs. Where the experiment was carried to the F_2 and F_3 generations, it was found that the offspring of the fertile half all bred true to fertility, while those of the sterile half all persisted in having 50 per cent sterile and 50 per cent fertile. The ratio here, as the investigator points out, is 1:1. As to the value of the results obtained, I am not in a position to judge. However, the fact that the investigator is dealing with the haploid generation instead of the usual diploid generation lends a new interest to the matter and opens up a possible new field of research.

Thus far I have attempted to give a general idea of some of the work done heretofore upon sterility in pollen, and its relation to hybridization. The work of DEVRIES and his "Mutationstheorie" resulting therefrom have given a great impetus to a search for the true means of evolution in living matter. The Darwinian idea that species arise through a gradual loss or gain of characters and the survival of the fittest of these has been very seriously attacked. The "sporting" condition which DARWIN recognized as occurring sometimes, but which he considered to be of far less importance than the changes brought about gradually, has been made to play a very much more important part by DEVRIES' mutation idea.

DEVRIES bases his theory primarily upon the much discussed species *Oenothera Lamarckiana*, which he had been growing for several generations in his garden, and of whose origin he is in no way certain. As I have already shown, sterile pollen, though not necessarily present in hybrids, is a common character. Recently JEFFREY (21) has shown that, so far as his examination has extended, morphologically sterile pollen does not occur, except in a percentage small enough to be accounted for by unfavorable conditions, in plants which are either monotypic or are isolated either in a geographical sense or through the time in which the flowers

are matured. He has shown, moreover, that below the angiosperms a comparatively small amount of sterility occurs, and that in the gymnosperms it is almost entirely absent. On the other hand, the angiosperms present a very different aspect. Certain families of the last have long been a puzzle to the systematist. BATESON (**22**) points out that in *Oenothera Lamarckiana* a large percentage of sterile pollen is present. Other geneticists and cytologists, including DEVRIES himself, have noted this, but perhaps BATESON was the first to attribute it to hybridization. JEFFREY (**21**) has shown that the condition of abortive pollen is characteristic not only of *Oenothera Lamarckiana*, but of the *Oenothera* species in general, and that, moreover, the entire family of the Onagraceae is so characterized. Because of this he considers that DEVRIES' "Mutationstheorie," in so far as it is based upon *Oenothera Lamarckiana*, is lacking in justification. In other words, in order to establish such a theory securely, one must deal with plants and with animals which are species in the fullest sense of the word. Geneticists have long recognized the variability of hybrids, and hence plants with any trace of hybridization should not be used as a basis for a theory of such fundamental importance as that promulgated by DEVRIES. In a recent article DEVRIES (**23**) has attempted to refute JEFFREY'S contention that pollen sterility is a criterion for hybridization. He states that, from such a viewpoint, we might consider the abortion of the 3 mother cells in the formation of the mature egg, which customarily takes place in the angiosperms, as a criterion for their hybrid origin. Again, he cites the case of certain sedges in which ordinarily 3 of the pollen mother cells abort and we find their remnants clinging to the mature pollen grain. His examples appear to me to be chosen from conditions which have no direct relation to the situation found by JEFFREY.

After what I have just said it is fitting, perhaps, to add a few words as to what really helps to make up a true species. In attempting such a discussion I realize that I am treading upon more or less forbidden ground; however, I shall give views which seem to me to be well supported by the observations of experts. There are those who would separate the idea of species entirely from that of a

hybrid, but, on the other hand, we find a large number of prominent biologists who hold a different view. In an article by MEEHAN (**24**) I find the following reference to LINNAEUS: "The great father of botany does not seem to have much, if indeed any, idea of sterility in his comprehension of the term. He believed that a large number of plants which he regarded as true species and to which he gave specific rank and specific names originated as hybrids. Thus we have *Trifolium hybridum*, *Trigonella hybrida*, *Campanula hybrida*, etc., among a large number of similar Linnean names." OLIVER (**25**) remarks that "natural hybrids have been known for a long time and we have every reason to believe that they have existed when and where conditions were favorable for their production." Recently GATES (**26**), in an article upon *Oenothera Lamarckiana*, writes as follows: "Crossing must have taken place in the ancestry of *Oenothera Lamarckiana* as well as in other flowers which are open pollinated. Among open pollinated plants (and the same is probably true for animals) there is no such thing as a 'pure' species, but, rather, many interbreeding races, whose combinations vary from generation to generation, make up the population." Upon the animal side views similar to this have been expressed. In an article recently published GEROULD (**27**) has shown how important a rôle hybridization has shown in forming species, and its relation to mutation.

I might cite other references (such as BATESON **22**, JANCZEWSKI **28**, CASTLE, BACOT, PROUT, etc.) to show how various biologists consider the term species. However, perhaps the foregoing will be sufficient to give some idea of the difference in opinion concerning the matter. Suppose we grant that two such ideas of a true species can exist together, then are the angiosperms, or at least many of them, species in the same sense as those which we find among the gymnosperms? In other words, is not the greater abundance of angiosperms as compared with the number of gymnosperms to be accounted for, to a large extent, through the formation of natural hybrids which breed true and have characters distinct enough to make them good species when viewed from the systematic standpoint? Certain families like the Onagraceae, Violaceae, Solanaceae, Rosaceae, etc., have long presented great difficulties to botanists.

Recently a specialist in *Oenothera* (BARTLETT **29**) has published an article in which he makes what he calls "12 elementary species of *Onagra*," 5 of which are made out of our common evening primrose (*Oncothera biennis*). This is surely a perplexing condition. BRAINERD (**30**), who has made a very careful study of the species of *Viola*, both in the field and under controlled conditions, has met with a very disconcerting situation. He finds a difference in the classes of hybrids. When hybrids are made between nearly related but distinct species, they have characters intermediate between the unlike characters of the parent forms. They are stable, breeding true from generation to generation, and showing a very vigorous growth, but are always more or less sterile. On the other hand, when crosses are made between species which are more or less doubtfully distinct, or between races, or between a species and a variety, there is usually quite a different result. In the first place, the offspring of such crosses have not intermediate characters, but recombinations of the unlike characters of the parent forms. As a result, the individual plants are dissimilar, some reverting to one or the other parent form, and others presenting the "sporting" characteristics. Another very important characteristic difference is that we do not usually find the fertility damaged either in the first or in subsequent generations. He speaks of these as "the so-called Mendelian hybrids, the despair of the systematist but the vantage ground of the breeder of new and useful 'varieties.'" Finally, he cites the instance of several species of *Aquilegia* which he allowed to grow indiscriminately together, and which in a short time gave off 5 hybrids. This case is similar to that of the violets which, owing to the greater amount of the cleared land, can be greatly increased in range and hence have a much greater opportunity for hybridization.

In connection with BRAINERD'S remark concerning his so-called Mendelian hybrids it seems well to quote a few lines from another. CORRENS (**31**) was one of the first to rediscover MENDEL. He evidently from the first appreciated certain limits to the scope of MENDEL'S laws.

Inzwischen hat mir ein Aufenthalt in den Alpen Gelegenheit gegeben, eine Anzahl von Bastarden zwischen unzweifelhaften Arten zu untersuchen

(*Cirsium palustre*×*spinosissimum*, *Cirsium heterophyllum*×*spinosissimum*, *Achillea moschata*×*nana*, *Achillea macrophylla*×*moschata*, *Carex echinata*×*foetida*, ferner *Melandryum album*×*rubum*, hier eine vollkomme gleitende Reihe von einer Art zu andern). Dabei ist mir zweifelhaft gebleiben, auch nur bei einzigen dieser Bastarde in einem einzigen Merkmalspaar ein wirkliches Dominiren verkommt, ganz sicher ist, dass in fast allen Punkten, in denen die Eltern differiren, der Bastard die Merkmale beider Eltern zeigt, jedes abgeschwächt, wenn auch in verscheidenem Grade.

Other paragraphs might be cited which bear more or less upon the subject, but I shall cite but the closing line of the article.

Die Aufdeckung der Mendel'schen Regeln wird also kaum dazu beitragen, dass von jetzt ab Speciesbastarde und Rassenbastarde in einem Topf geworfen werden, und man statt dessen nur von Mono- Di- etc., Polyhybriden sprechen wird, sie wird im Gegentheil wohl der Anfang für eine schärfere Trennung der beiden sein.

The Rosaceae, which is the last of the families mentioned as being a perplexing problem for the systematist, has among its many genera several whose species are very much unsettled. Of these *Crataegus*, *Rosa*, and *Rubus* may be mentioned as distinctive. With these 3 genera there have been great changes wrought within the last few years. Taking *Crataegus*, for example, we find a very interesting account of its present condition by BROWN (**32**). When GRAY'S *Field, forest, and garden botany* was published in 1857 there were listed in it but 12 species and 2 varieties. Again, in GRAY'S *Manual of botany* of 1867 we find only 10 species and 4 varieties given. It was not until after the publication of CHAPMAN'S *Flora of the southern United States* in 1897, which gave but 15 species within its range, that we find any great increase in the number of species. Since then many species have been described. In fact, since 1896 there have been, according to BROWN, 864 species and 18 varieties described. Where will this species making end? As BROWN states, "Why did not the systematists discover this great number of species before? Have they been recently hybridizing? Undoubtedly a part of the great increase may be explained by a more intensive study upon the part of the systematic botanist, but does this account for it all?" BRAINERD, in reply to BROWN'S inquiry, states that "the Rosaceae of all orders is the *most predisposed to hybridize. Rosa, Rubus, Geum, Amelanchier,*

and *Malus* are notorious for the forms resulting from interbreeding. By analogy we should expect the same condition of things in *Crataegus*."

Rubus presents a condition of things quite like that of *Crataegus*. Although GRAY'S *Manual* of the last edition contains 38 species all told, yet the authors carefully state that in *Eubatus* we have "a group of great taxonomic difficulty, in which many species have been recently proposed. Of these the better marked have been here freely included, but without entire confidence that the future intensive study may not show them to be intergradient and perhaps in many cases hybrid forms." Because of this great variation and because of the uncertainty as to which are and which are not true species, I have selected this genus in which to make a careful study of the pollen conditions. However, before proceeding to the results of my research, it seems best to give a brief historical account of *Rubus* and the work done upon it up to the present time.

Rubus, like *Crataegus*, has had an almost miraculous growth in number of species within a few years. Only 18 years ago GRAY'S *Manual* recognized but 3 species and 2 varieties for New England, and, as BRAINERD (**33**) remarks, 2 of these specific names were wrongly applied. In 1898 BAILEY (**34**) published his *Evolution of native fruits* in which he recognized 13 species of *Rubus* of the *Eubatus* group for New England. Since that time BLANCHARD has published detailed descriptions of 40 or more forms, most of which he has considered as species. Many of these, as I shall show later, appear to be of hybrid origin from a study of the pollen; but from an external study alone he came to the conclusion that they were worthy of specific rank.

In 1911 BICKNELL (**35**), in an article entitled "Have we enough New England blackberries?" makes the following statement: "*The least unstable species (here) possess some kind of ready pliancy which answers often with marked emphasis to slightly changed conditions of growth, and further, all the species by some freely practised method of versatility acquire variously in combination with their own proper characters the features of associated members of the group.*"[2] He adds that these facts impart an extraordinary natural variability

[2] The writer is responsible for the italicizing.

and undoubtedly also *a facility in hybridizing which is, perhaps, not exceeded in any of our flora*. In speaking of BLANCHARD'S species he remarks that "probably 60 per cent of these are synonyms, while the remainder, with possibly a few exceptions, *appear to disclose themselves as scarcely doubtful hybrids*." In his mind there are about 11 species of *Eubatus* which alone can be considered valid, while the others are hybrids. He also gives a list of their names as he believes they should appear. BRITTON, in the new edition of the *Illustrated flora*, recognizes but 10 species, none of BLANCHARD'S naming being mentioned even as synonyms. More recently RYDBERG in his monograph of *Rubus* has allowed 27 of the *Eubatus* group for the northeastern United States, including 7 of BLANCHARD'S naming and 4 of BAILEY'S. Of the remaining 30 and more of BLANCHARD'S names, 9 are disposed of as synonyms and 24 as hybrids.

BRAINERD (**33**) in his recent article upon *Rubus*, which he has published after years of study and from which I have drawn many of my historical data, remarks: "No mere herbarium botanist is qualified to disentangle the perplexing intricacies of a group of plants like *Rubus*. The days of random guessing at parents are past or should be." He then states what to him are the proper methods of procedure in determining a true species, and gives a list of 9 "probable good species" in Vermont.

The phenomenon of variability in *Rubus* appears not to be confined to American species, but to be characteristic of the genus everywhere. HOOKER in his *Flora of the British Isles* names 41 forms as species, while GARKE'S *Flora von Deutschland* contains more than 60 species. FOCKE (**5**) makes the following interesting statement:

> Die Gattung *Rubus* ist ungemein formenreich und zerfällt in eine Anzahl verscheidener wohl charakterisirter Untergattungen. Einige Gruppen bestehen aus äusserst zahlreichen nahe verwandten Racen, welche die Grenzen zwischen den durch wesentliche Charactere gescheidene Arten oft völlig vermischen. Auch viele sonst gut umgrenzte Arten sehr formenreich natürliche Hybride scheinen innerhalb vieler Artengruppen leicht zu entstehen, wahrscheinlich gehen haufig aus ihnen neue constante Racen hervor.

During the summer of 1915 I was able to obtain from the Harvard Botanical Garden and from the Arnold Arboretum buds

of about 40 species of *Rubus*. In every case I picked those buds which were about to open, and in this way I was able to obtain pollen grains which were mature. If these were morphologically good, they were large, round, and very full of protoplasmic substances. Material, on the other hand, in which the pollen had not matured normally showed it to be shrunken and empty, having lost all or most of its protoplasm and having but a vestige of the nucleus present.

During the following winter I made a study of the pollen condition present in these buds, the method in most instances being as follows. Owing to the fact that perfectly formed pollen is very densely filled with protoplasmic substances, no harm resulted from killing directly with 95 per cent alcohol. Other more tedious methods were tried, but no better results were obtained. The buds were then pricked, the air pumped out, and the buds thoroughly dehydrated in absolute alcohol. After this they were placed in a 2 per cent solution of celloidin dissolved in equal parts of synthol and ether. The grades of celloidin were raised 2 per cent every 12 hours, until 16 per cent was reached. Then small pieces of dry celloidin were added at 12- or 24-hour intervals, until a sufficient density was reached. When such a condition was obtained, the buds were removed one at a time and allowed to harden in chloroform for about 12 hours. They were then placed in equal parts of 95 per cent alcohol and glycerine. When completely hardened these were mounted upon blocks in a 6 per cent solution of celloidin and cut upon the microtome. After being cut and stained in Haidenhain's iron alum hematoxylin and counterstained in safranin, they were mounted in balsam. Such a method allows the observer actually to see the pollen as it occurs in the anther, and by so doing it gives one a more accurate idea of the true condition.

The genus *Rubus* in the last edition of GRAY'S *Manual* is divided into 5 groups with FOCKE as an authority for their names. Of the first group, which is called the IDAEOBATIS group, I have 3 different forms (*Rubus strigosus* Michx., *R. neglectus* Peck, and *R. occidentalis* L.). It is here made a very small group, having but 3 well authenticated species. *R. strigosus* Michx. (fig. 1) is given as a synonym

for a variety of *R. idaeus* L. I find the pollen condition to be nearly perfect and hence to give every indication that, from a morphological basis at least, the plant should be given a specific rank. *R. neglectus* Peck (fig. 2), on the other hand, shows a large percentage, perhaps 50 per cent, of sterile pollen. This condition goes very well with the remark made in the *Manual* that it probably is a self-perpetuating hybrid. Fig. 3 is an enlarged view of a portion of the field shown in fig. 2. *R. occidentalis* L., which is the well known black raspberry or thimbleberry, is given a specific rank in the *Manual* and the pollen condition indicates that such a position is tenable.

Of the second, or ANAPLOBATUS group. I have 2 members. The first, *R. odoratus* L. (fig. 4), the beautiful flowering raspberry, shows pollen almost universally good. Such a position would be expected from the late period in which it bears its flowers. Even should it be susceptible to crossing with other members of the genus, its lateness in regard to the opening of its buds would make it very hard to do so. *R. parvifolius* Nutt., on the other hand, although it is put here as a good species, contains a large percentage of pollen which is clearly sterile.

I have been unable to obtain any material of the members of the third (CHAMEMORUS) and of the fourth (CYLACTIS) groups, including in the *Manual* but 3 species.

It is the fifth or EUBATUS group which is by far the largest and has given the most trouble. In studying these I have found several whose pollen is nearly perfect, so nearly so in fact that the few shrunken grains which do appear may be accounted for by some unfavorable physiological condition. By far the greater number, however, show a greater or less degree of sterility. For convenience I have divided these last into those members ranging from sterility of about 25 to about 75 per cent; and also those which show a range greater than 75 per cent.

Among those with good pollen appear the following: *R. alleghaniensis* Porter, *R. setosus* var., *R. fruticosus* L., and *R. amnicolus* Blanch. *R. alleghaniensis* Porter (fig. 5) and *R. setosus* Bigel. (fig. 6) are given specific rank in the *Manual*. *R. fruticosus* L. (fig. 7), which is a European form, is suggested here as perhaps the

same as *R. laciniatus* Willd., with the leaves less cut. *R. amnicolus* Blanch. (fig. 8) is placed together with *R. orarius* Blanch. as a synonym for *R. pergratus* Blanch.

Of those with a percentage of over 25 and less than 75 sterile I have *R. frondosus* Bigel., *R. recurvans* Blanch., *R. glandicaulis* Blanch., *R. Jecklyanus* Blanch., *R. orarius* Blanch., *R. arundelanus* Blanch., and *R. amabilis* Blanch. Of these GRAY'S *Manual* cites 4 as probably of specific rank, namely, *R. frondosus* Bigel. (figs. 9 and 10), *R. recurvans* Blanch., *R. glandicaulis* Blanch., and *R. Jecklyanus* Blanch. *R. orarius* Blanch. is placed together with *R. amnicolus* Blanch. as a synonym for *R. pergratus* Blanch. *R. arundelanus* Blanch. is considered as a synonym for *R. recurvans* Blanch. Finally, *R. amabilis* Blanch. appears under the same name as *R. canadensis* L. Comment upon this group is unnecessary, unless to remark upon the apparent perplexity its members have caused systematists. Clearly from the standpoint of sterility much hybridization is now occurring and has already taken place.

Of the last group, those with over 75 per cent of the pollen sterile, I have 4 specimens. These are *R. biformispinus* Blanch. (fig. 11), *R. Andrewsianus* Blanch. (fig. 12), *R. tardatus* Blanch. (fig. 13), and *R. peculiaris* Blanch. These all seem to have distinctive form, and from the standpoint of the systematist may warrant a species rank, at least they are so considered in the *Manual*. However, the pollen condition plainly points not only to hybridization, but to hybridization between species which must have been quite distinct and certainly quite incompatible.

Besides these forms I have 10 or more which are not included with the range of GRAY'S *Manual*. But one of these, *R. deliciosus*, (fig. 14), appears to be a good species when taken from the standpoint of absence of sterile pollen. I have *R. caesus turkestanicus*, *R. corylifolius*, *R. spectabilis*, *R. plicatifolius*, *R. multiformis* (fig. 15), *R. semierectus*, and *R. spectabilis plena* (fig. 16). One of the forms, *R. nobilis* (figs. 17 and 18), which is known as a hybrid, is almost entirely sterile, and, as one would expect, contains but a very few grains which can possibly be considered as viable.

Since my purpose has not been to attempt to place systematically the members of the genus *Rubus*, I have not given any

account of the various characters upon which the systematists separate its species. Indeed it appears to be too hard for the systematist himself. Scarcely any two systematists agree as to which are and which are not true species. This is especially well shown by the large number of synonyms which one meets in any systematic work upon the genus.

However, I have found that sterility of pollen is a common characteristic throughout the entire genus. But few of the species indicate a perfect pollen condition and some are almost entirely sterile. In other words, the genus *Rubus* indicates to me a condition which appears to prevail in many angiosperms. JEFFREY (**21**) has already pointed out how prevalent sterile pollen is throughout the whole family of the Onagraceae, and other groups of plants are now being worked upon in this laboratory which seem to show a similar condition.

In closing, it seems to me that many of the species of the angiosperms are in a very different condition from that of others of the same group and from practically all those of the gymnosperms. They appear often to be natural hybrids which are formed, as BRAINERD has pointed out, as a result of a cross between distinctly separate species. Such crosses do not result in the segregation which Mendelian crosses usually exhibit, but rather in the blending of the characters of the parents. CORRENS (**31**) in his observations has noted such a situation in regard to certain plants. Such offspring may breed true to their respective characters and thus perpetuate a distinct form which, from a systematic point of view, is entirely of specific rank, but which should be treated by the plant breeder in an entirely different manner. Such forms usually have a large percentage of sterile pollen. Thus *Rubus* hybrids formed by distinct species are very sterile, in fact almost entirely so, while crosses of varieties which are closely related, and which hence are usually more compatible, may give almost no indication of their hybrid origin.

Thus it appears to me that the genus *Rubus* is one of those angiospermic genera in which the term "species" must be used in a different sense from that applied to many other members of the angiosperms, and practically to all of the gymnosperms. It is a

group which is a puzzle to the systematist, and whose species will, perhaps, never be definitely established.

Conclusions

1. Sterility of pollen has long been recognized as a criterion of hybridization.

2. Crosses between distinct species have long been known to be more or less sterile and to behave differently from crosses between more closely related forms or varieties.

3. True species when crossed do not, in most cases, follow the laws of MENDEL, but tend rather to blend to form more or less constant types, often systematically recognized as species.

4. Many species of the angiosperms are species in a very different sense from those of the lower plants and of the gymnosperms in particular.

5. In some cases they are natural hybrids which have external characteristics distinct and constant enough to have specific rank from a systematic standpoint.

6. Although these species may be distinct from the systematic standpoint, yet they must be treated in a different manner from the standpoint of the evolutionist and the plant breeder.

7. Finally, the species of the genus *Rubus*, as shown from the pollen condition and also from external characters, clearly hybridize very frequently in nature, giving rise to constant forms often recognized as true species.

In closing I wish to express my sincere thanks to those in charge of the Arnold Arboretum and of the Harvard Botanical Garden for the privilege of collecting. I desire, also, to express my thanks to Miss DAY, librarian of the Gray Herbarium, for assistance in securing literature, and to others who have kindly aided me in carrying out my purpose. This work has been carried on in the Laboratories of Plant Morphology of Harvard University under the direction of Professor E. C. JEFFREY, and to him I am indebted for advice and assistance.

HARVARD UNIVERSITY

LITERATURE CITED

1. CAMERARIUS, R. J., Über das Geschlecht der Pflanzen; translated from "De sexu plantarum epistola." 1694 (Ostwald's Klassiker der exakten Wissenschaften **105**:49. 1899).
2. KÖLREUTER, J. G., Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen. Leipzig. 1760 (OSTWALD'S Klassiker der exakten Wissenschaften **41**:39. 1893).
3. KNIGHT, A., Observations on hybrids. Trans. Hort. Soc. London **4**:367. 1821.
4. HERBERT, W., On the production of hybrid vegetables. Trans. Hort. Soc. London **4**:15. 1820.
5. FOCKE, W. O., Die Pflanzenmischlinge. p. 489. 1881.
6. DUTROCHET, M., The sterility of hybrid plants. Gard. Mag. **8**:500. 1832.
7. GÄRTNER, C. F. V., Bastarderzeugung. Stuttgart. 1849.
8. DUCHARTE, P., Sur l'hybridité dans les végétaux. Ann. Sci. Nat. Bot. **19**:125. 1863.
9. MENDEL, G., Versuche über Pflanzen-Hybriden. Vehr. Naturf. Vereins Brünn 4. 1865.
10. ROSENBERG, O., Über die Tetradenteilung eines *Drosera*-Bastardes. Ber. Deutsch. Bot. Gesells. **47**:53. 1904.
11. DETLEFSEN, J., The fertility of hybrids in a mammalian species cross. Amer. Breed. Mag. **3**:261–264. 1912.
12. JUEL, H. O., Beiträge zur Kenntnis der Tetradenteilung. Die Tetradenteilung bei einer hybriden Pflanze. Jahrb. Wiss. Bot. **35**:638. 1900.
13. TISCHLER, G., Über Embryosackobliteration bei Bastardpflanzen. Bot. Centralbl. Beih. **15**:408–420. 1903.

———, Über Entwicklung des Pollens und der Tapetenzellen bei *Ribes*-Hybriden. Jahrb. Wiss. Bot. **42**:545. 1906.

———, Über die Entwicklung der Sexualorgane bei einen sterilen *Byronia*-Bastarden. Ber. Deutsch. Bot. Gesells. **24**:83–96. 1906.

———, Zellstudien an sterilen Bastardpflanzen. Arch. Zellforsch. **1**:33–151. 1908.

14. GEERTS, J. M., Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von *Oenothera Lamarckiana*. Receuil Trav. Bot. Néerl. **5**:93. 1909.
15. BELLING, JOHN, The mode of inheritance of semi-sterility in the offspring of certain hybrid plants. Zeitsch. Ind. Abst. Vererbungs. **12**:303–342. 1914.
16. OSAWA, I., Cytological and experimental studies in *Citrus*. Jour. Coll. Agric. Tokyo **4**:83–116. 1912.
17. DORSEY, M. J., Pollen development in the grape with special reference to sterility. Minn. Agric. Exp. Sta. Bull. 144. 1914.
18. BRAINERD, E., Reference found in BATESON'S *Mendel's Principles of heredity*, p. 284. 1909.

19. JESENKO, F., Sur un hybride fertile entre *Triticum sativum* et *Secale cereale*. Conf. Internat. Génétique. Paris. 1913. Rep. 4. p. 391.
20. SUTTON, A. W., Experiments in crossing wild peas from Palestine with commercial peas. Conf. Internat. Génétique. Paris. 1913. Rep. 4. p. 358.
21. JEFFREY, E. C., The mutation myth. Science N.S. **39**:488–491. 1914.
———, Spore conditions in hybrids and the mutation hypothesis of DEVRIES. BOT. GAZ. **58**:322–336. 1914.
———, Some fundamental morphological objections to the mutation theory of DEVRIES. Amer. Nat. **49**:5–22. *figs. 6*. 1915.
22. BATESON, W., MENDEL'S principles of heredity. 1909.
23. DEVRIES, H., The coefficient of mutation in *Oenothera biennis* L. BOT. GAZ. **59**:169–196. 1915.
24. MEEHAN, THOMAS, Fertility in hybrids. Gard. Chron. N.S. **22**:363–372. 1884.
25. OLIVER, G. W., Hybridization. Amer. Gard. **29**:397–400. 1889.
26. GATES, R. R., Mutation in *Oenothera*. Amer. Nat. **45**:577–606. 1911.
27. GEROULD, J. H., Species building by hybridization and mutation. Amer. Nat. **48**:321–338. 1914.
28. JANCZEWSKI, E., Bull. Acad. Cracovie, no. 6. p. 24. 1889; p. 288. 1892.
29. BARTLETT, H. H., Twelve elementary species of *Onagra*. Cybele Columbiana **1**:37–56. 1914.
30. BRAINERD, E., Hybridization in the genus *Viola*. Rhodora **8**:6, 49. 1906.
31. CORRENS, C., Über Levkojenbastarde, zur Kenntnis der Grenzen der Mendel'schen Regeln. Bot. Centralbl. **84**:97–113. 1900.
32. BROWN, H. B., The genus *Crataegus* with some theories concerning the origin of its species. Bull. Torr. Bot. Club **37**:251–260. 1910.
33. BRAINERD, E., The blackberries of Vermont. Vt. Bot. Club Bull. 9. April 1914.
34. BAILEY, L. H., Evolution of native fruits. 1898.
35. BICKNELL, E. P., Have we enough New England blackberries? Bull. Torr. Bot. Club **37**:393–403. 1910.

EXPLANATION OF PLATES X–XII

FIG. 1.—*Rubus strigosus* Michx.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 2.—*R. neglectus* Peck: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 3.—*R. neglectus* Peck: more highly magnified view of fig. 2; ×300.

FIG. 4.—*R. odoratus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 5.—*R. alleghaniensis* Porter: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 6.—*R. setosus* Bigel.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 7.—*R. fruticosus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 8.—*R. amnicolus* Blanch.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 9.—*R. frondosus* Bigel.: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 10.—*R. frondosus* Bigel.: more highly magnified view of fig. 9; ×300.

FIG. 11.—*R. biformispinus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 12.—*R. Andrewsianus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 13.—*R. tardatus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×600.

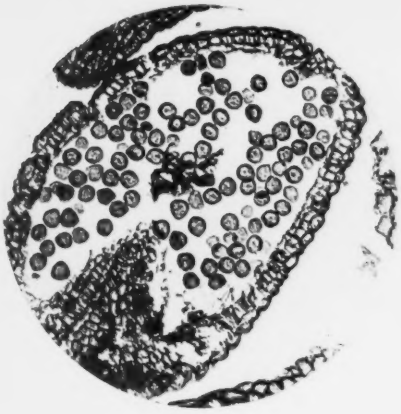
FIG. 14.—*R. deliciosus:* transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 15.—*R. multiformis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 16.—*R. spectabilis plena:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 17.—*R. nobilis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 18.—*R. nobilis:* more highly magnified view of fig. 17; ×300.



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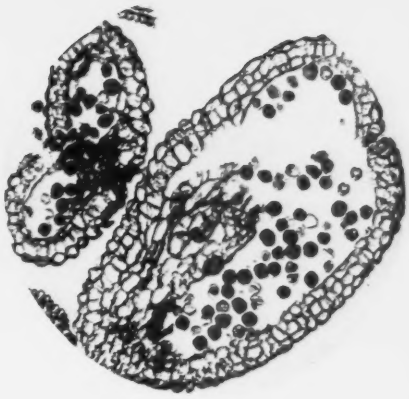

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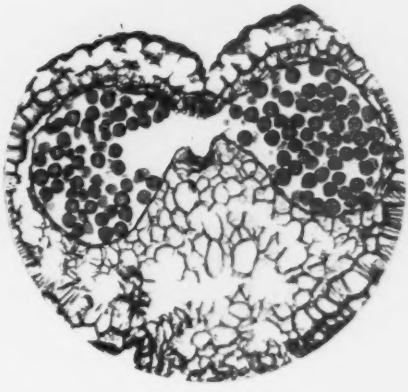

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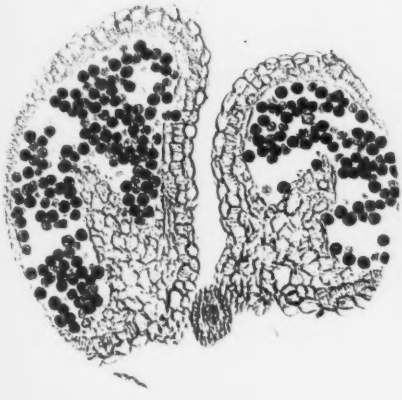

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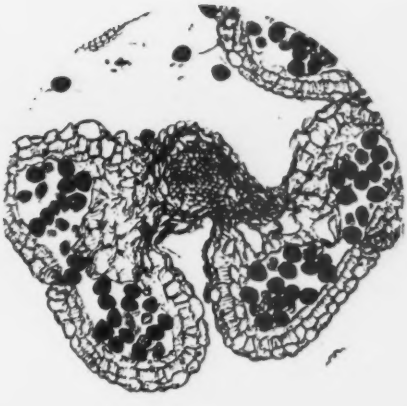

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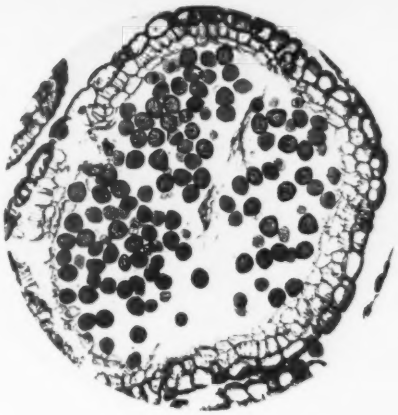
HOAR on RUBUS



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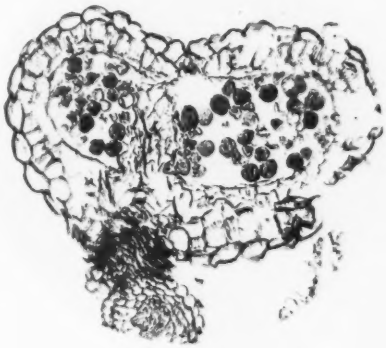

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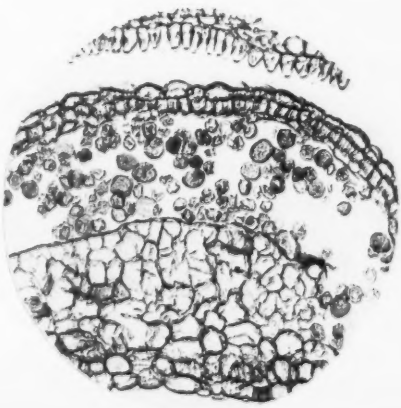

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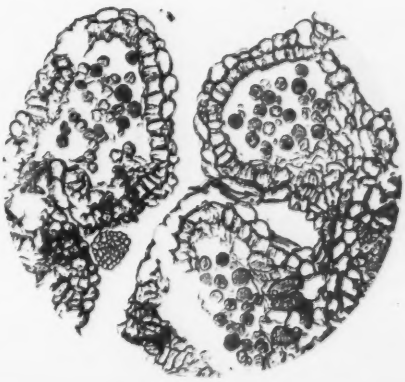

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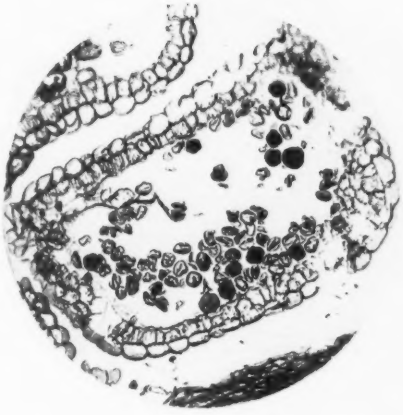

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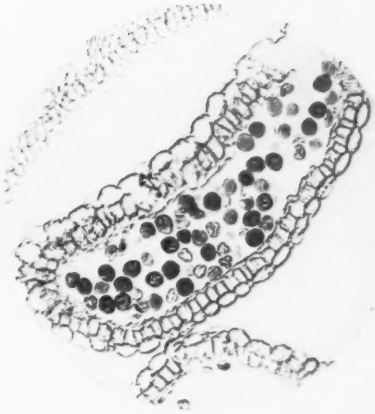

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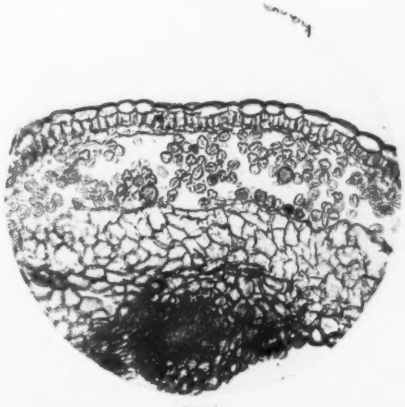

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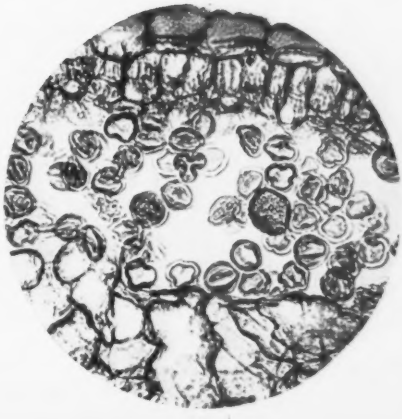

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HOAR on RUBUS

FURTHER OBSERVATIONS ON CHLORANTHY IN DROSERA INTERMEDIA

MICHAEL LEVINE

(WITH PLATE XIII AND THREE FIGURES)

NAUDIN (15) was among the first to record a new method of propagation other than by seed for the genus *Drosera*. He described the appearance of buds of two young plants on the dorsal surface of a mature leaf of *Drosera intermedia*. These buds appeared between the midvein and the margins of the leaf. KIRSCHLEGER (8) made similar observations in the case of *D. capensis*. He noted buds arising from the epidermal cells at the apex or near the base of the leaf, or on the petiole itself. This species was later studied by WINKLER (19), who confirmed the observations of KIRSCHLEGER. NITSCHKE (14) described vegetative budding for *D. rotundifolia* from material collected in the field. The buds are described as appearing on the dorsal surface of the leaf, and were most commonly found in the early fall. Others have confirmed and extended the observations of NITSCHKE and the earlier data to which I have referred. GROUT (7) ascribes the appearance of these buds to excessive moisture conditions. GRAVES (6) in 1896 collected and placed plants of *D. rotundifolia* in an artificial bog. He found that a number of small plants appeared on the upper surface of old leaves, and in some cases these plants appeared from the ventral surface and edges of the leaf as in *Bryophyllum*. LEAVITT (9, 12) was able to propagate *D. filiformis*, *D. binata*, and *D. dichotoma* from cut leaves. He noticed that after placing leaves of the species named in sphagnum for a period of about 3 weeks new plants appeared from adventitious buds, which finally grew to maturity. He likewise observed that leaves, first formed from such buds in plants of *D. binata*, were orbicular like those of *D. rotundifolia;* while leaves coming from buds on *D. filiformis* were like those of *D. intermedia*. These observations, together with other studies on reversion (LEAVITT 10), led him to

the conclusion that the leaf of *D. rotundifolia* is the original type of leaf from which those of other species have arisen. AMES (1) showed the possibility of propagating *Drosera* species from old leaves cut from mature plants. He worked with *D. filiformis*, *D. intermedia americana*, *D. rotundifolia*, and *D. binata*. He noticed that when mature leaves fell on the sand in which these plants were growing, adventitious buds appeared. The condition most favorable to the development of these buds in his opinion is a low temperature.

DIXON (2), like AMES and LEAVITT, was able to obtain adventitious buds on leaves of *D. rotundifolia*. He differs with GROUT in that he asserts that adventitious buds appear only when the parent plant is allowed to dry out. He found that under these conditions the leaves of *D. rotundifolia* produce adventitious buds on the dorsal surfaces after 2 months. He also found that new plants may arise in the axils of leaves and between the petiole and main axis of the inflorescence as axillary buds. GOEBEL (4) showed further that a portion of a leaf of *D. binata* could produce adventitious buds. He cut an arm of a leaf of *D. binata* and placed it on a moist substratum. After a time he observed the appearance of adventitious buds whose leaves were rounded and similar to those of *D. rotundifolia*, and he states that these leaves agree in shape with those of the young plants of *D. binata*.

Miss ROBINSON (17) repeated the work of LEAVITT, AMES, and GOEBEL, with similar results. She describes the appearance of roots on the young adventitious buds which appeared on leaves of *D. rotundifolia* placed in sphagnum. She also found that leaves placed with the dorsal surface down developed buds on the upper or ventral surface. Miss ROBINSON holds that these adventitious buds are in no way connected with the vascular system of the parent plant.

SALISBURY (18) found the appearance of adventitious buds in *D. rotundifolia* and *D. intermedia* Hayne. Signs of these buds appear on the leaves of these 2 plants in the late spring of the year following their removal to the greenhouse. The age of the leaf which bears these buds may vary. The first leaf rudiment appears as a small protuberance on the dorsal surface of the leaf. This is

followed by another leaf rudiment opposite the first, and the next leaves follow in succession, forming a rosette-like structure in the axils of the leaves first formed. As against Miss ROBINSON, KIRSCHLEGER and WINKLER, SALISBURY maintains that sections through these buds show that they are connected with the fibro-vascular bundle of the leaf on which they appear. Salisbury believes that these buds form a definite reproductive mechanism in these species of plants.

GOEBEL (5) reports on *D. pygmaea* the appearance of a highly specialized type of brood body resembling in shape the gemmae of *Marchantia*. These bodies appear in rosettes and are dorsi-ventral. The under surface of each body is smooth, while the upper surface is horseshoe-shaped. Stomata and vascular bundles are present. GOEBEL believes that each brood body arises from a leaf *anlage*.

PLANCHON (16) described an anomaly in the flowers of *D. intermedia*. He asserts that the ovary becomes elongated and the carpels bear both tentacles and ovules. All gradations between an ovule and a tentacle were found. The petals of these flowers were likewise modified, being leaflike in nature and bearing tentacles. PLANCHON asserts that all organs become chloranthic except the calyx. FERNALD (3), while collecting bog plants, found a form of *D. rotundifolia* in which the inflorescence was subcapitate and consisted of a few flowers. The petals and sepals were greenish to crimson in color, and the ovary instead of producing a normal capsule formed a rosette of glandular foliage leaves. This tendency was noted in other floral organs. LEAVITT (11) studied these flowers and states that they are quite sterile. FERNALD makes no mention of stamens. It seems that they are generally absent, and this constitutes the main difference between this form and the one described later. The constancy of this abnormality in *D. rotundifolia* led FERNALD to make a new variety which he called *D. rotundifolia comosa*. Neither FERNALD nor LEAVITT gives figures of these modified flowers. FERNALD regards them as a means of vegetative propagation.

In studying the development of pollen my attention was attracted by the appearance of a number of plants of *D. intermedia*.

These were growing in the water, and at the apex of the flower stalk a head of tentaculiferous flowers was borne. Again, in August 1915 I found in the same locality a number of these plants. They were growing in the water of a shallow arm of Horicon Lake, Lakehurst, New Jersey. The plants were of the long-stemmed type such as has been figured by NITSCHKE (**14**) for *D. rotundifolia*, and extended about 4–5 in. above the surface of the water. Associated with them was a *Castalia* species, and the banks of the bog were covered with sphagnum in which *D. rotundifolia* and *D. filiformis* grew in great abundance. Normal plants of *D. intermedia* were present also. These abnormal inflorescences resemble in all respects those described by FERNALD (**3**) for *D. rotundifolia*. The plants were not so numerous; in fact, the monstrosity appeared in comparatively few plants. As the case is quite a striking one, I have thought it worth while to figure and describe the abnormal flowers in some detail. Herbarium specimens have been deposited in the New York Botanical Garden.

The plants bearing the abnormal flowers were in all other respects like the normal *D. intermedia*. Under the conditions in which they were growing the exposed leaves and their tentacles were reddish. The long stem and delicate roots were submerged and buried in the mud. The apical portion of the stem bears an imperfect rosette of leaves. Here also appears the short, thick flower stalk on the summit of which the abnormal heads of greenish flowers are found. On superficial examination (text figs. 1–3), the small headlike inflorescences seem to be mere clusters of diminutive leaves. More careful study shows that the cluster is really a group of chloranthic flowers. The term "chloranthy," as used here, indicates that all or nearly all of the organs of the flower assume the appearance and form of leaves (**13**). Each flower is commonly subtended by 3 bracts. These bracts are narrow, lanceolate, leaflike structures which may have small sessile glands. All the parts of the flower except the stamens have become leaflike. This is a more extreme case of chloranthy than that described by PLANCHON (**16**). The chloranthic flowers of an inflorescence are in different stages of development, the size of the flower generally indicating its relative age. The largest I found

were 6–8 mm. in diameter, and the smallest were 1–2 mm. in diameter. There is no correspondence between the degree of development of the flower and its position in the inflorescence. They seem to be promiscuously scattered over the slightly thickened end of the flower stalk.

There are 5 sepals as in the normal flowers. They are slightly green and have a number of tentacles as shown in fig. 2. The marginal tentacles are generally larger and as a rule vary in number

FIG. 1

from 2 to 4. The surface tentacles have smaller stalks and are very often lacking. In one case I found 2 adjacent fused sepals as shown in fig. 1, which represents the ventral surface of the calyx showing the attachment of the sepals. The tentaculiferous petals alternate with the sepals. While in the normal flower they are usually white or pinkish in color, in the chloranthic flowers they are pale green, and, as noted, covered with a varying number of normal tentacles (fig. 3). The shape of these petals is like that of the foliage leaf with the characteristic spatulate blade and a rather long, narrow, and flattened petiole. The tentacles are scattered quite regularly along the margin and over the surface

of the blade. The marginal ones are larger. Inside this whorl of petals there are 5 stamens. These appear to be in every respect normal and compare favorably in size and shape with the stamens in normal flowers. Normal tetrad pollen grains have been found in their anthers (fig. 4). In the case (fig. 5) of one flower which is mentioned later, the filaments were broader and the anthers in some cases consisted of a single locule each, yet all intermediate stages between unilocular and bilocular anthers were present. In these anthers no pollen was found. Unlike PLANCHON'S (**16**) chloranthic flowers of *D. intermedia*, these had no carpels or any indication of carpels (figs. 6, 13). The center of the flower was occupied by a cluster of leaf rudiments in various stages of development, as described by FERNALD (**3**) and LEAVITT (**11**) for *D. rotundifolia* leaf. The outer leaf rudiments are best developed, while the ones toward the center are progressively smaller (fig. 6). In older flowers these rudiments have become fully developed spatulate blades resembling those of the normal vegetative leaf. In a single capitate inflorescence all stages in the development of the central leaf rudiments can be found. Fig. 7 represents a small chloranthic flower in such a head. The sepals are fully developed, while the petals, although they are fully expanded, have not all extended their tentacles. The unfolding leaflike petals show the characteristic incurved form common to the vegetative leaves. The stamens in this flower (fig. 7) are normal and are few in number. The carpels as such are entirely lacking, but are replaced by a small number of very minute leaf rudiments, of which one is considerably larger than the others. Figs. 8 and 9 represent larger chloranthic flowers. The sepals shown in fig. 8 are larger and much broader than the average I observed. In the center of the flower there is a large rosette of young leaves entirely replacing the carpels (fig. 8). The outer leaf rudiments are large and almost ready to unfold. Fig. 9 represents a chloranthic flower in which the leaves are slightly farther advanced. Fig. 10 represents a chloranthic flower in which the central leaves have attained the greatest development I found. The flower measured approximately 6 mm. in diameter. Only one petal and sepal are figured, but the usual number 5 was present here also. The outer whorl of the apical leaf group has fully

developed into 4 comparatively large spatulate leaves covered with numerous tentacles and having a well developed vascular system (compare modified sepals, petals, and leaves in fig. 10). The central rosette of young leaves is large and in all appearances resembles the normal winter buds common to *D. intermedia*.

The cases figured represent what appears to be the ordinary structure of the chloranthic flowers in this species of *Drosera*, but variations from this type can be found. Fig. 11 represents a

FIG. 2

flower in which some of the leaves in the petal series have few tentacles. Fig. 12 represents one of a cluster of 6 flowers in a capitate inflorescence in which an abnormality appeared. In this flower the number of sepals is reduced to 2; they have tentacles and are like those previously described. The number of petals is likewise reduced. The stamens are 5 in number and are characterized by having broad filaments (fig. 5) with anthers, as just noted, consisting of 1 or 2 locules in which no pollen grains were found. The leaf rudiments resemble those in the ordinary chloranthic flowers. Although I have not observed their further development, there can be no doubt that these chloranthic flowers serve as a

means of vegetative propagation. The central rosette of leaf rudiments is comparable to a diminutive form of winter bud, such as is common to this and other species of the *Drosera*. The appearance of tentacles and especially chlorophyll on the petals of the chloranthic flowers may be an adaptive feature to enable the flower to maintain itself until rooted in the substratum.

FERNALD'S (3) monstrous flowers in *D. rotundifolia comosa* appear to be the most marked case of chloranthy described for the

FIG. 3

genus. The organs of the flowers of this species may all become modified and assume the appearance of leaflike structures. It seems that vegetative propagation is absolutely essential to the perpetuation of this form. PLANCHON'S case of chloranthy in *D. intermedia* shows flowers with organs least modified. The calyx in these flowers never becomes modified and the normal carpels may also be present. The stamens and the petals are invariably modified. In the case observed all the organs except the stamens are modified to assume the form of tentaculiferous leaves. The carpels are replaced by a rosette of leaves resembling

a winter bud. The degree of chloranthy is more developed than in the case described by PLANCHON, and appears also to be more constant, although abnormalities among the monstrosities occur. It appears from these cases that the degree of chloranthy may vary. It may be argued that a case like the one described in the present paper is less abnormal than the other cases mentioned. It appears that normal pollen may be formed, and under favorable conditions cross-pollination may take place between these forms and the normal species of *Drosera;* that is, sexual reproduction has not entirely disappeared. At the same time, these plants appear to be capable of propagating themselves vegetatively by the chloranthic flowers which may function as buds. The case is perhaps parallel to the adventitious buds on the leaves.

The conditions which favor the development of these flowers are not evident. It may be, as suggested by GROUT for adventitious buds, that an abundance of moisture is very essential. It seems from FERNALD'S observations and my own that chloranthic flowers in *Drosera* species are formed only in plants which grow in water. The bearing of such cases of reversion of flowers to vegetative buds on our general conception as to the relation of sexual and asexual reproduction is not very clear. It appears, however, that under unfavorable conditions vegetative reproduction replaces in part, if not altogether, the sexual method. This is contrary to our general conception of reproduction, since it is commonly conceded that under favorable conditions vegetative reproduction prevails almost entirely to the exclusion of sexual reproduction.

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LITERATURE CITED

1. AMES, O., An easy method of propagating *Drosera filiformis*. Rhodora 1:172. *pl. 8*. 1899.
2. DIXON, H., Adventitious buds in *Drosera rotundifolia*. Notes from the Botanical School of Trinity College, Dublin 144–145. 1901.
3. FERNALD, M. L., A peculiar variety of *Drosera rotundifolia*. Rhodora 7:8, 9. 1905.
4. GOEBEL, K., Einleitung in die experimentelle Morphologie der Pflanzen. 1908 (p. 196, *fig. 97*).

5. GOEBEL, K., Morphologische und biologische Bemerkungen. 18. Brutknospenbildung bei *Drosera pygmaea* und einigen Monokotylen. Flora **98**:324–335. 1908.
6. GRAVES, J. A., Notes on *Drosera*. Plant World **1**:28. 1897.
7. GROUT, A. J., Adventitious buds on leaves of *Drosera rotundifolia*. Amer. Nat. **32**:114–115. 1898.
8. KIRSCHLEGER, M., Note sur quelques anomalies végétales. Bull. Soc. Bot. France **2**:722–724. 1855.
9. LEAVITT, R. G., Adventitious plants of *Drosera Rhodora* **1**:206–208. *pl. 10*. 1899.
10. ———, Reversionary stages experimentally induced in *Drosera intermedia*. Rhodora **5**:265–272. 1903.
11. ———, Translocation of characters in plants. Rhodora **7**:13–20, 21–31. 1905.
12. ———, Seedlings and adventitious plants of *Drosera*. Torreya **9**:200–203. 1909.
13. MASTERS, M. T., Vegetable teratology. London. 1869.
14. NITSCHKE, TH., Wachsthumsverhältnisse des rundblättrigen Sonnenthaues. Bot. Zeit. **18**:57–61, 65–69. *pl. 2*. 1860.
15. NAUDIN, Note sur des bourgeons nés sur une feuille de *Drosera intermedia*. Ann. Sci. Nat. Bot. **14**:14–16. *fig. 1*. 1840.
16. PLANCHON, J. E., Sur la famille des Droséracées. Ann. Sci. Nat. Bot. III. **9**:79–99. *pls. 5, 6*. 1848.
17. ROBINSON, W. J., Reproduction by budding in *Drosera*. Torreya **9**:89–96. 1909.
18. SALISBURY, E. J., On the occurrence of vegetative propagation in *Drosera*. Ann. Botany **29**:308–310. 1915.
19. WINKLER, H., Über regenerative Sprossbildung auf den Blättern von *Torenia asiatica* L. Ber. Deutsch. Bot. Gesells. **21**:96–107. 1903.

EXPLANATION OF PLATE XIII

All figures were drawn with the aid of a Leitz hand lens ocular 10.

CHLORANTHIC FLOWERS OF DROSERA INTERMEDIA HAYNE

FIG. 1.—Ventral surface of a calyx of a chloranthic flower in which 2 sepals have fused.

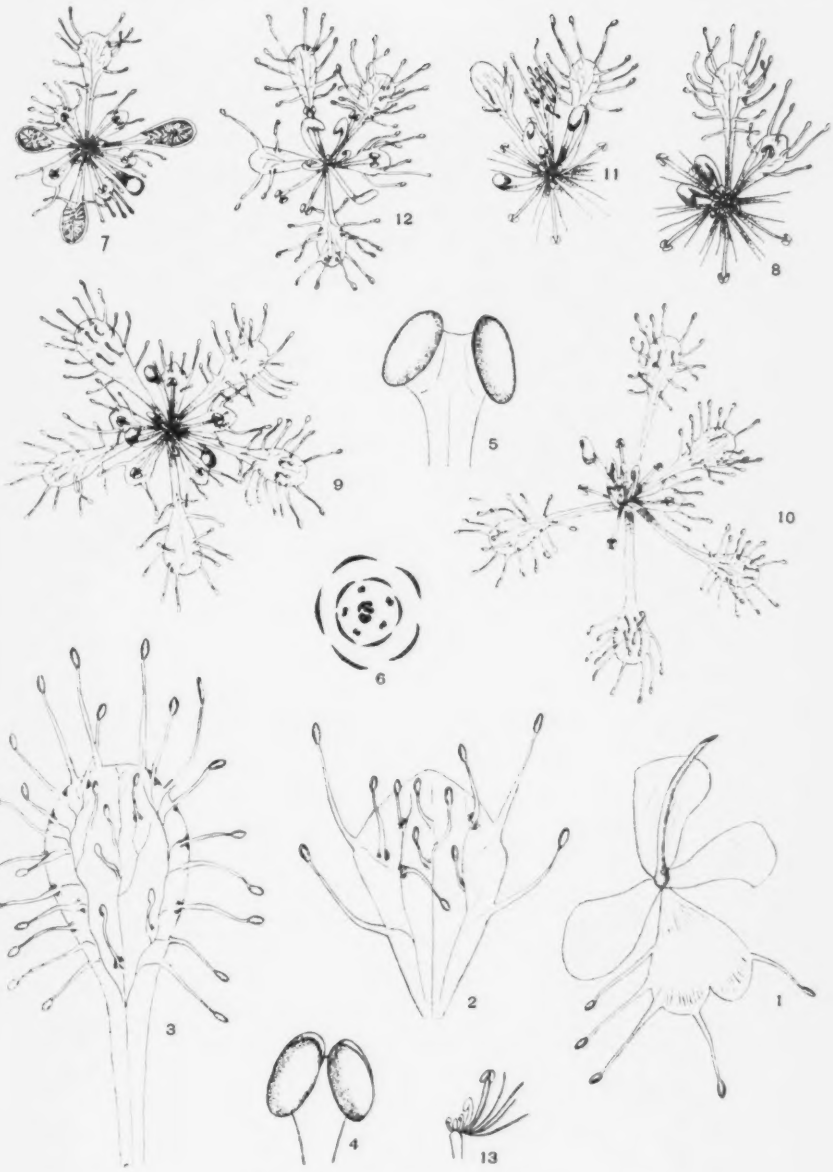
FIG. 2.—Dorsal surface of a sepal of a chloranthic flower covered with tentacles.

FIG. 3.—Dorsal surface of a petal of a chloranthic flower showing numerous tentacles.

FIG. 4.—Normal stamen of a chloranthic flower in which normal pollen is borne.

FIG. 5.—Abnormal stamen in which no pollen is found.

FIG. 6.—Schematized cross-section of chloranthic flower.



LEVINE on CHLORANTHY

FIG. 7.—Young chloranthic flower in which the leaflike petals are not yet fully developed; the carpel is replaced by a number of leaf rudiments.

FIG. 8.—A slightly older stage; sepals very large.

FIG. 9.—Another slightly older stage.

FIG. 10.—An older stage in which outer whorl of leaf rudiments are developed into leaflike organs resembling normal spatulate leaf of *D. intermedia*.

FIG. 11.—Variation of a chloranthic flower in which the number of tentacles on the petals is relatively small.

FIG. 12.—Another abnormal chloranthic flower in which the number of sepals and petals is reduced to 2 and 3 respectively and the stamens are abnormal, as in fig. 5.

FIG. 13.—Schematized longitudinal section of a chloranthic flower.

A CONTRIBUTION TO OUR KNOWLEDGE OF SPLACHNIDIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 220

MABEL LEWIS ROE

(WITH PLATES XIV–XVIII)

Introduction

Splachnidium rugosum Grev. is a monotypic genus of more than ordinary interest because investigators have placed it in one group or another according as some particular feature seemed more worthy of emphasis. The earliest mention of this plant was by LINNAEUS (**9**) as *Ulva rugosa;* a few years later SUHR (**18**) described it as *Dumontia rugosa;* and in 1830 GREVILLE (**3**) founded the genus *Splachnidium*, retaining the specific name already given.

Most authors have placed it with the Fucaceae, but in 1892 MITCHELL and WHITTING (**10**) published an account which was incorporated in 1895 by MURRAY (**11**) in his book. This account includes the morphology of both vegetative and reproductive tissues. As a result of their investigations, these authors felt justified in establishing a new family, making the following statement (p. 9): "The sum of the characters of *Splachnidium* so expressly excludes it from any existing natural order that there is no other course open to us than to establish one for its reception under the name of Splachnidiaceae." KJELLMAN'S account (**6**) restored it to the Fucaceae; and in 1904 OLTMANNS (**13**) placed it with the Ectocarpaceae under the subgroup Encoelieae.

The material for this investigation was secured by Professor CHARLES J. CHAMBERLAIN at Glen Cairn, near Cape Town, South Africa, on February 28, 1912. A second collection was made at the same place on August 30, 1914, by Miss EDITH STEPHENS of South African College, Cape Town, and sent to Professor CHAMBERLAIN. The work was undertaken with the hope of determining (1) the nature of the contents of the reproductive sacs, whether zoospores or gametes; and (2) the origin of the so-called "apical cell," a

strikingly characteristic feature of *Splachnidium*. The material proved inadequate for the study of reproduction, but furnished a good series for the development and fate of the "apical cell." No mitosis was observed, and hence no chromosome count was possible. It is hoped that some one in the region where *Splachnidium* grows may be interested enough to investigate material secured at the exact time when cell division occurs, probably at night, or to observe the behavior of the living bodies after discharge from the reproductive sac.

Origin of the initial row

The first feature which tends to set the initial cells apart from adjacent border tissue is the taking on of a heavy coat of mucilage (fig. 1). In this and subsequent early stages the heavy mucilage coat is the only character which distinguishes the initial from young vegetative hairs abundant in the apical region (figs. 2–5). A second feature marking this unique structure is the beginning of resorption of cross-walls and loss of original definite nuclear structure (figs. 4, 6, 13, 15). Gradually a complete linear row of cells, reaching entirely through the thallus, is differentiated from the surrounding tissue as an initial row (figs. 5, 7, 8, 13, 15).

Sometimes there is no evident differentiation of tissue at an early stage, but portions of old apical hairs may assume the heavy mucilage coat (figs. 9–11), and there follows an involving of the entire linear row of which the hair is a terminal portion. Part of the old hair may be cast off, eventually leaving only the lower portion (fig. 11).

By the time an entire linear row is so differentiated, the characteristic filiform appendage, which has been described (**10**) as "growing between the cells of the thallus toward the center of the branch," begins to be evident (figs. 15, 16). Pressure of abutting tissue has narrowed the basal portion into a tail-like process as resorption of old walls and cell contents has gone on, whereas freedom from pressure above has allowed expansion into a pear-shaped oval. At this stage all of the original contents left in the filiform portion consist of granular patches of cytoplasm, and in the oval portion there is a similar granular substance with the

addition of darkly staining bodies; there is no trace left of nuclear structure.

As active growth continues on each side of the initial row, rapid division occurs in the external layers of the thallus, whereas the inner cells, lying on the border of the central cavity, are pulled apart, not dividing to keep pace with the active outer tissue. This separation leaves the filiform basal process swinging loose, as it were, in the central cavity (figs. 17–20). The entire structure lies at the base of a slight depression, the result of arrested growth of the initial row followed by activity of abutting tissue.

Such, then, is the history of the initial row. *It is the result of more or less disintegration of an entire linear row of the thallus*, with perhaps the addition of portions of a terminal hair. *The exact amount of tissue originally involved*, whether including a terminal hair in part or in entirety, *determines the length and size of the mature structure.* For a long time the writer was puzzled by the great variation in length displayed by these bodies, and the explanation was apparent only when their origin was completely worked out.

The structure described in the foregoing is found in connection with any activity of the plant, as growth at the main apex, or production of lateral branches, or inception of conceptacles.

The conceptacle

In the formation of the conceptacle, the cavity in which the initial row lies becomes deepened as more and more of the abutting border tissue is involved. *Rapid radial division of these border cells contributes new tissue to the conceptacle at the same rate and in the same way as ordinary thallus tissue is developed.* In the early stages of the conceptacle, except for the initial row, there is no essential difference between adjacent tissue of the thallus and that of the actual cavity of the conceptacle (fig. 19). Presently, however, the lining cells of the conceptacle become elongated, and subsequently, by transverse division at the basal end, develop into septate hairs (figs. 20, 21). This behavior is exactly as noted by the writer in a recent investigation of *Fucus* (**14**) as the stage in the history of the conceptacle known as the "hair pit."

Some of the hairs, as in *Fucus*, are shed later and the lining cells put forth new papillate growths which elongate into sacs (fig. 22) which are ultimately filled with spherical bodies (fig. 24), variously described as zoospores (**10, 11**) and gametes. AGARDH (**1**), HARVEY (**4**), HOOKER (**5**), and LAING (**8**) all describe *Splachnidium* as dioecious, although no one of these authors reports having actually observed antheridia. LAING speaks of oogonia "obscurely pedicelled," developed from the lining of the conceptacle, each oogonium giving rise "to a large number of oospheres, thus differing from all other Fucaceae that have hitherto been described. Each oosphere is very small, compared with the oospheres of other Fucaceae." In another paragraph he states that "as antheridia have never been observed it is just possible that these hairs [apical hairs] may be antheridial in function." The writer's feeling is that the reproductive sacs contain isogamous gametes. However that may be, the conceptacles, at the time the reproductive bodies are ready for discharge, still contain the "initial" as a conspicuous feature (fig. 23).

Discussion

MURRAY (**11, 12**) and also MITCHELL and WHITTING (**10**) speak of the "modified cell" of *Splachnidium* as homologous with the "initial" of *Fucus;* and yet these same authors make this "unique cell" one of the reasons for separating *Splachnidium* from other families. The similarity holds true only in so far as the conceptacle is concerned, where, as previously shown by the writer (**14**), the conceptacle in each genus is the result of arrested development and partial breaking down of a portion of the external layer of the thallus followed by great activity of abutting tissue. The initial row is in no way comparable to the true apical cell of *Fucus*, segments of which are the basis of all tissue of the thallus.

The fact that the initial row of Splachnidium occurs wherever there is great activity is additional evidence for the simple origin of the conceptacle. *Splachnidium* is a particularly favorable plant for study along this line, since the same structure accompanies ordinary vegetative growth as well as reproductive activity.

In establishing a new family MITCHELL and WHITTING (10) summarize their reasons in the following statement (p. 8):

> While, then, the characteristics of *Splachnidium rugosum* are such that it can be placed in no existing natural order of the Phaeophyceae, its nearest allies appear to be the Fucaceae on the one hand and the Laminariaceae on the other. In its vegetative structure, in the nature of its thallus, and in the existence of conceptacles, *Splachnidium* bears a resemblance to the Fucaceae; it differs from them, however, in its mode of growth, the former increasing by means of an apical meristem, while the growth of the latter is due to division of an apical cell. In the presence of an apical meristem *Splachnidium* approaches the Laminariaceae, but at the same time there is no plant in this order which has a cell corresponding to the remarkable cell found at the apex of the main axis and lateral branches of *Splachnidium*. In its reproduction it is allied to the Laminariaceae, and the production of sporangia within conceptacles might seem to point to a narrower limitation of the fertile sorus of plants of this order, recalling the difference between perithecium and apothecium in the fungi.

As to vegetative growth, the tissue of the thallus of *Splachnidium* bears an equal resemblance to the Fucaceae and the Laminariaceae, the only essential difference being in origin. *Splachnidium* and the Laminariaceae are products of a meristem, the former apical, the latter intercalary, according to the evidence of most authorities, and not apical as described by MITCHELL and WHITTING in the passage just quoted. The Fucaceae, too, are characterized by apical growth of the thallus, but all tissue is the actual result of segmentation of a definite apical cell. In vegetative growth, then, *Splachnidium* is intermediate between the two groups.

The presence of conceptacles certainly seems a definite connecting link with the Fucaceae; and yet some of the Laminariaceae show a tendency to approach this method of reproduction. SMITH and WHITTING (17) describe deep furrows in *Macrocystis* and *Postelsia*, which closely resemble conceptacles in form and origin. In this feature, too, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae.

MURRAY (11, 12) and MITCHELL and WHITTING (10) conclude that the reproductive sacs of *Splachnidium* are neither oogonia nor antheridia homologous with those of the Fucaceae, but are sporangia homologous with those of the Laminariaceae, because of the

size of the organ, its unilocular nature, its single wall, the number of spores contained, the size of the spore, and the persistent empty case. On the basis of these features there is no reason why *Splachnidium* may not equally well be considered as having unilocular gametangia containing isogamous gametes. This would give to *Splachnidium* a primitive position among the Fucaceae, but would offer for the Phaeophyceae a link in the phylogenetic sequence comparable to the position occupied in the Chlorophyceae by such plants as *Ulothrix*.

Moreover, in the Fucaceae the oogonia and antheridia are unilocular, no permanent walls separating the gametes. Also DREW (**2**) has reported conjugation of isogamous gametes in *Laminaria digitata* and *L. saccharina*. "The resulting zygospore divides and gives rise to a chain of cells which may represent the $2x$ generation, and this in turn gives rise to the *Laminaria* plant, which represents the gametophyte, or x generation." His observations have as yet neither been verified nor disproved.

Recently SAUVAGEAU (**15, 16**) has concluded from his investigations that *Sacchorhiza* (and probably all Laminarias) presents a heterogamous sexuality with alternation of generations. The large plant is the sporophyte bearing uniform sporangia, and each sporangium develops like zoospores "which after transformation into embryospores become male gametophytes or female gametophytes of microscopic size and independent for life. The oosphere expelled from the female gametophyte, then fertilized, germinates at once and develops the plant of *Laminaria*."

In the contents of the reproductive sacs, therefore, as well as in form and origin of the conceptacles and in vegetative growth, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae. There seems to be no justification for establishing a separate family (**10**) with its main character "reproduction by spores contained in sporangia which are borne within conceptacles."

Further investigation will doubtless show a much more intimate connection between the Laminariaceae and Fucaceae than is at present recognized. *Splachnidium* may be placed with either group, since it has features common to both. Perhaps the presence

of definite and conspicuous conceptacles, even though scattered indefinitely over the entire thallus, places it more closely with the Fucaceae. There seems no good reason for placing *Splachnidium* (13) with the Ectocarpaceae.

Summary

1. The initial row of *Splachnidium* is similar in origin and development to the "initial" of the Fucaceae, except that in *Splachnidium* it involves an entire linear row of thallus tissue; it may or may not include a terminal hair; and it accompanies vegetative as well as reproductive activity.

2. It seems unwise to place *Splachnidium* under a separate family; rather is it preferable to retain it under the Fucaceae, regarding it as a primitive member of that group for the following reasons: (*a*) it closely resembles the Fucaceae in the structure of the thallus, but has an apical meristem in place of a segmenting apical cell with consequent dichotomy; (*b*) true conceptacles are present which in origin and development are of the same general type as those of the Fucaceae, but are scattered indefinitely over the entire plant body; (*c*) the reproductive sacs may prove to contain isogamous gametes.

Acknowledgment is due to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN for their suggestions and criticisms.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. AGARDH, J. G., Species, genera, et ordines Algarum. London. 1848.
2. DREW, G. H., The reproduction and early development of *Laminaria digitata* and *L. saccharina*. Ann. Botany **24**:177–190. *pls. 14, 15.* 1910.
3. GREVILLE, R. K., Algae Britannicae. Edinburgh. 1830.
4. HARVEY, W. H., Phycologia australica. London. 1858–1863.
5. HOOKER, J. D., Handbook of the New Zealand flora. London. 1867.
6. KJELLMAN, F. R., in ENGLER and PRANTL'S Die natürliche Pflanzenfamilien **1²**:Leipzig. 1897.
7. KÜTZING, F. T., Phycologia generalis. Leipzig. 1843.
8. LAING, R. M., Observations on the Fucoideae of Bank's Peninsula. Trans and Proc. New Zealand Inst. **18**:303–311. *pl. 10.* 1885.

9. LINNAEUS, C. VON, Mantissae Plantarum. Holmiae. 1767.
10. MITCHELL, M. O., and WHITTING, F. G., On *Splachnidium rugosum* Grev., the type of a new order of algae. Phyc. Mem. **1**:1–10. *pls. 1–3*. 1892.
11. MURRAY, G., Introduction to the study of seaweeds. London. 1895.
12. ———, On the cryptostomata of *Adenocystis*, *Alaria*, and *Sacchorhiza*. Phyc. Mem. **2**:59–63. *pl. 16*. 1893.
13. OLTMANNS, F., Morphologie und Biologie der Algen. Jena. 1905.
14. ROE, M. L., The development of the conceptacle in *Fucus*. BOT. GAZ. **61**:231–246. *pls. 14–17*. 1916.
15. SAUVAGEAU, C., Sur la sexualité hétérogamique d'une Laminaire (*Sacchorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:796–799. 1915.
16. ———, Sur les débuts du développement d'une Laminaire (*Sacchorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:740. 1915.
17. SMITH, A. L., and WHITTING, F. G., Notes on the sori of *Macrocystis* and *Postelsia*. Phyc. Mem. **1**:84–86. *pl. 20*. 1895.
18. SUHR, J. N. VON, Beiträge zur Algenkunde. Flora **24**:257–298. 1840.

EXPLANATION OF PLATES XIV–XVIII

All the drawings were made with the aid of the Abbe camera and reduced one-half in reproduction. For figs. 1–21, original magnification was 1580; for fig. 22, 790; and for figs. 23, 24, 1050.

FIG. 1.—Modified superficial cell, showing heavy coat of mucilage which distinguishes initial row from other border cells.

FIG. 2.—Two-celled stage of initial row.

FIG. 3.—Three-celled stage of same.

FIG. 4.—Two stages in development of initial rows; a two-celled stage at right and an older stage at left in which some of the cross-walls have been resorbed.

FIG. 5.—A linear row of the thallus, showing mucilage coat characteristic of an initial row.

FIG. 6.—An initial row in which cross-walls have been almost completely resorbed; traces of old walls still distinguishable.

FIG. 7.—A cut showing how initial row is usually terminated by sister cells, one of which usually fails to mature but sometimes initiates a young hair, as in fig. 8.

FIG. 8.—An initial row together with a young hair.

FIG. 9.—Basal portion of a branch of an apical hair which has become an "initial."

FIG. 10.—Lateral branch and basal portion of apical hairs which have become "initials."

FIG. 11.—Basal portion of an old apical hair in which cross-walls have been resorbed; the upper portion (unshaded) will be shed later.

FIG. 12.—An ordinary vegetative hair characteristic of apical region.

FIG. 13.—A completely involved linear row of the thallus with outer portion showing resorption of cross-walls and loss of nuclear detail.

FIG. 14.—Two initial rows which are modified young hairs.

FIG. 15.—An initial row including a linear row of the thallus together with a terminal hair; entire structure shows more or less disintegration and loss of cross-walls.

FIG. 16.—A stage which begins to show characteristic "filiform appendage" at basal end of initial row; almost all the cross-walls have been resorbed and original cell contents have disintegrated into granular patches.

FIGS. 17, 18.—Showing how interior cells of thallus pull away and release the "filiform appendage" from pressure.

FIG. 19.—A young conceptacle with young septate hairs.

FIG. 20.—A later stage of a conceptacle with development of septate hairs.

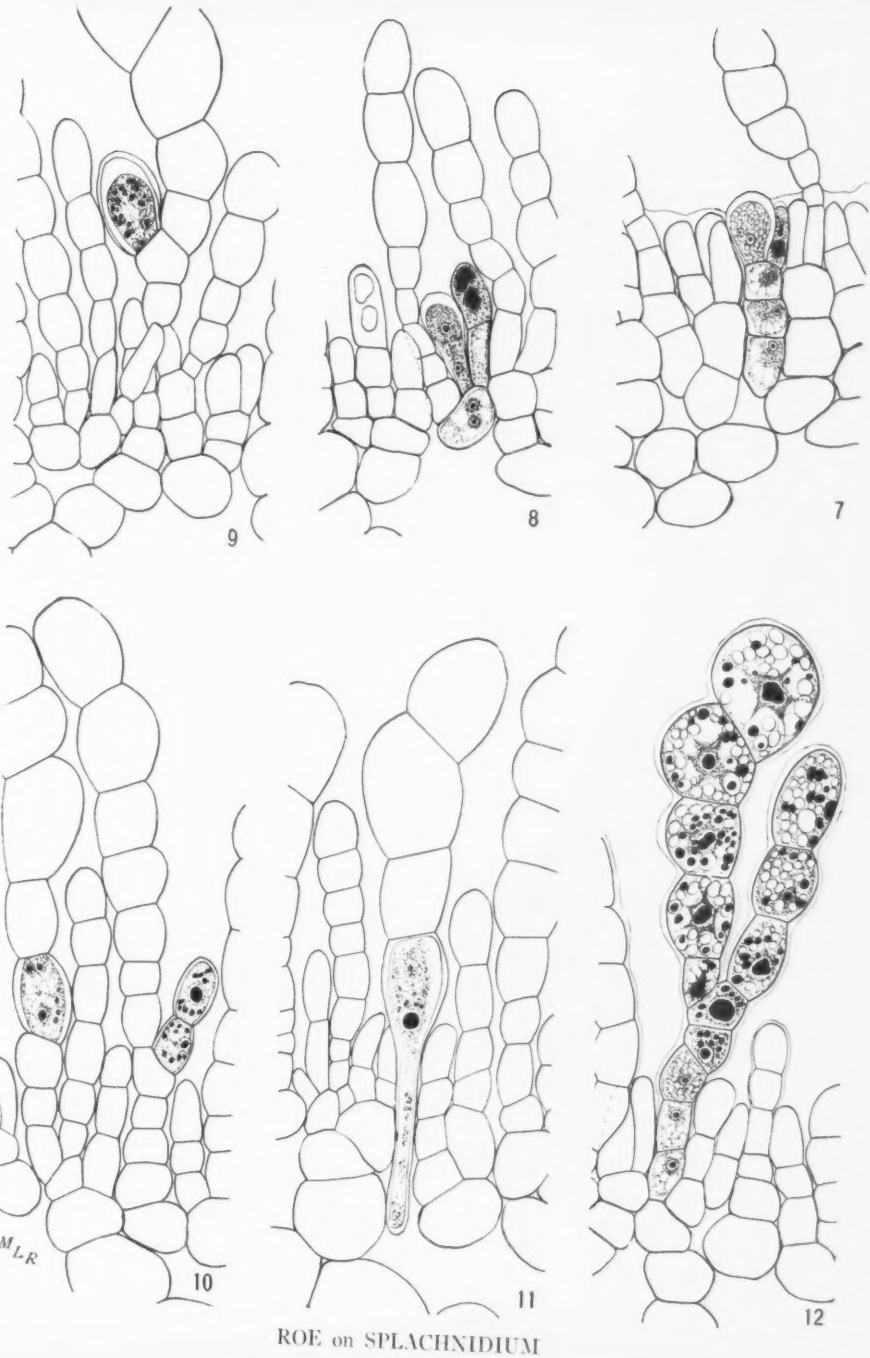
FIG. 21.—The hair pit stage of the conceptacle.

FIG. 22.—A mature conceptacle, showing various stages of reproductive sacs (gametangia?) and a few fragile hairs; old hairs have been shed.

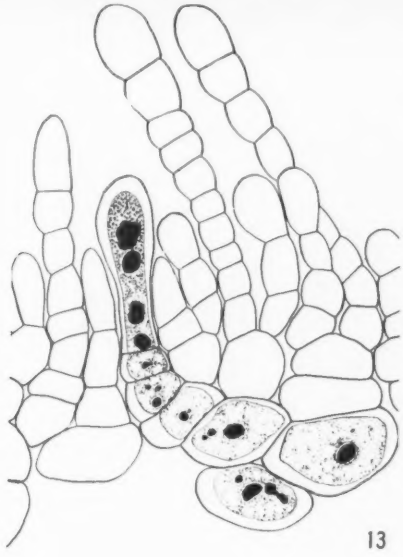
FIG. 23.—The initial row as it appears in a conceptacle at stage shown in fig. 22.

FIG. 24.—Gametangium (?) just before discharge of contents.

ROE on SPLACHNIDIUM



ROE on SPLACHNIDIUM



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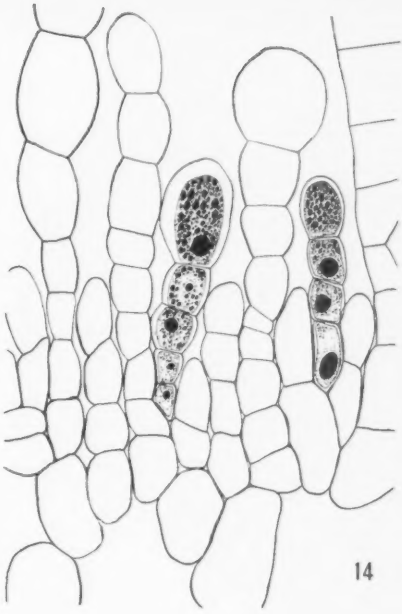

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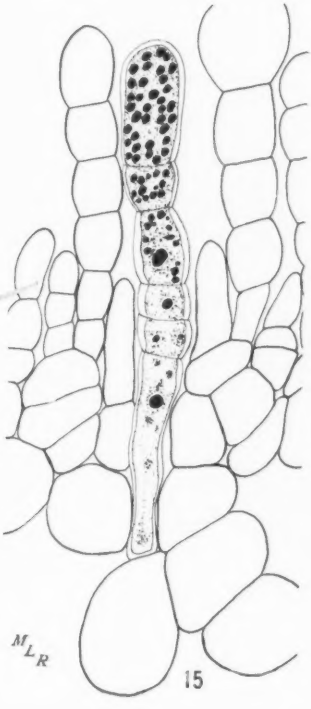

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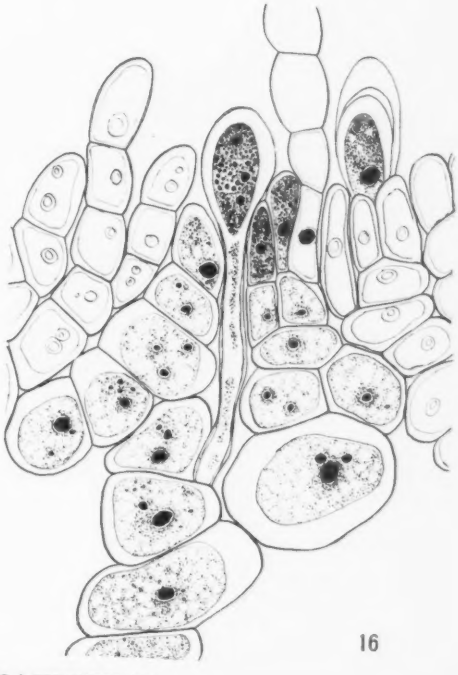

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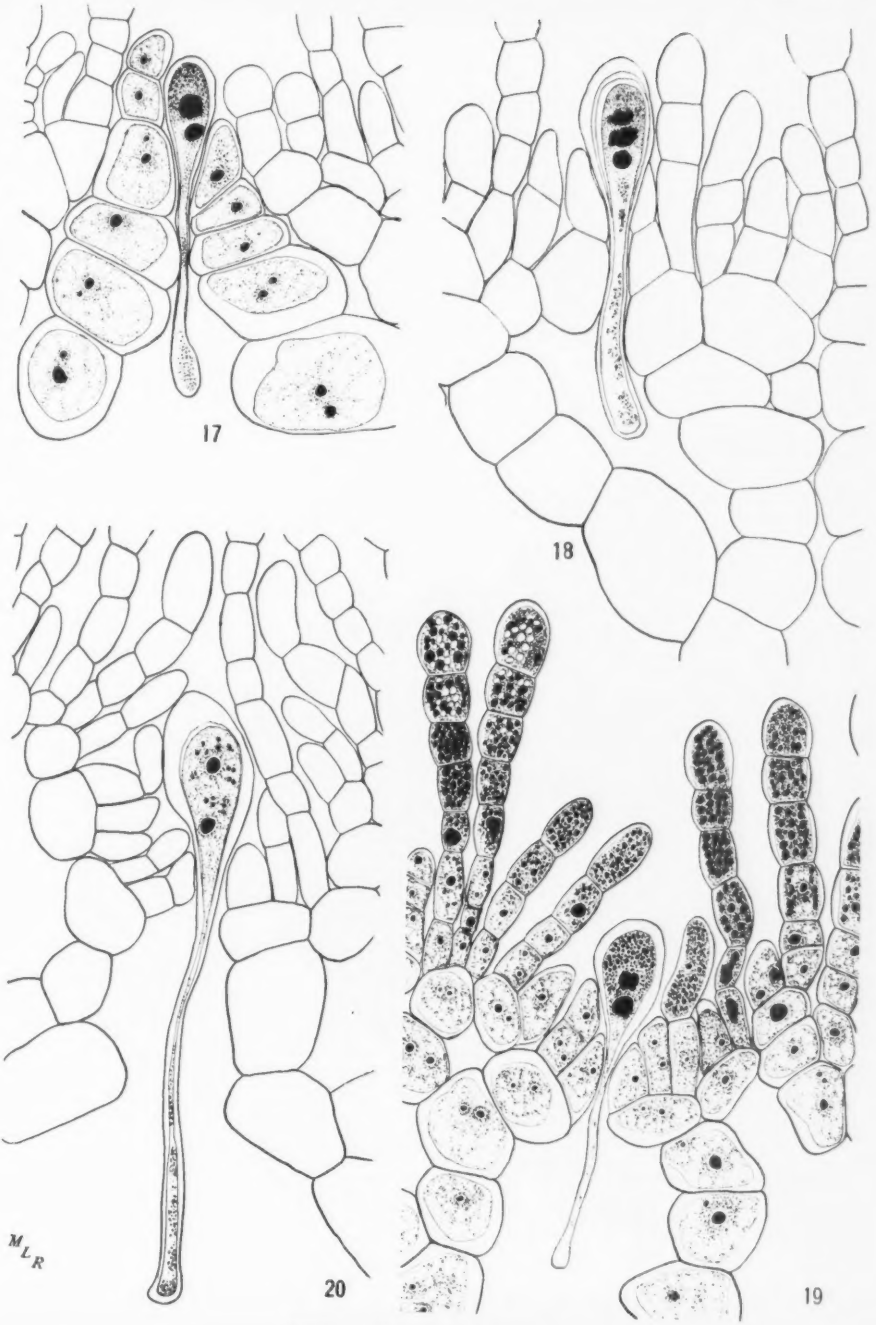
ROE on SPLACHNIDIUM



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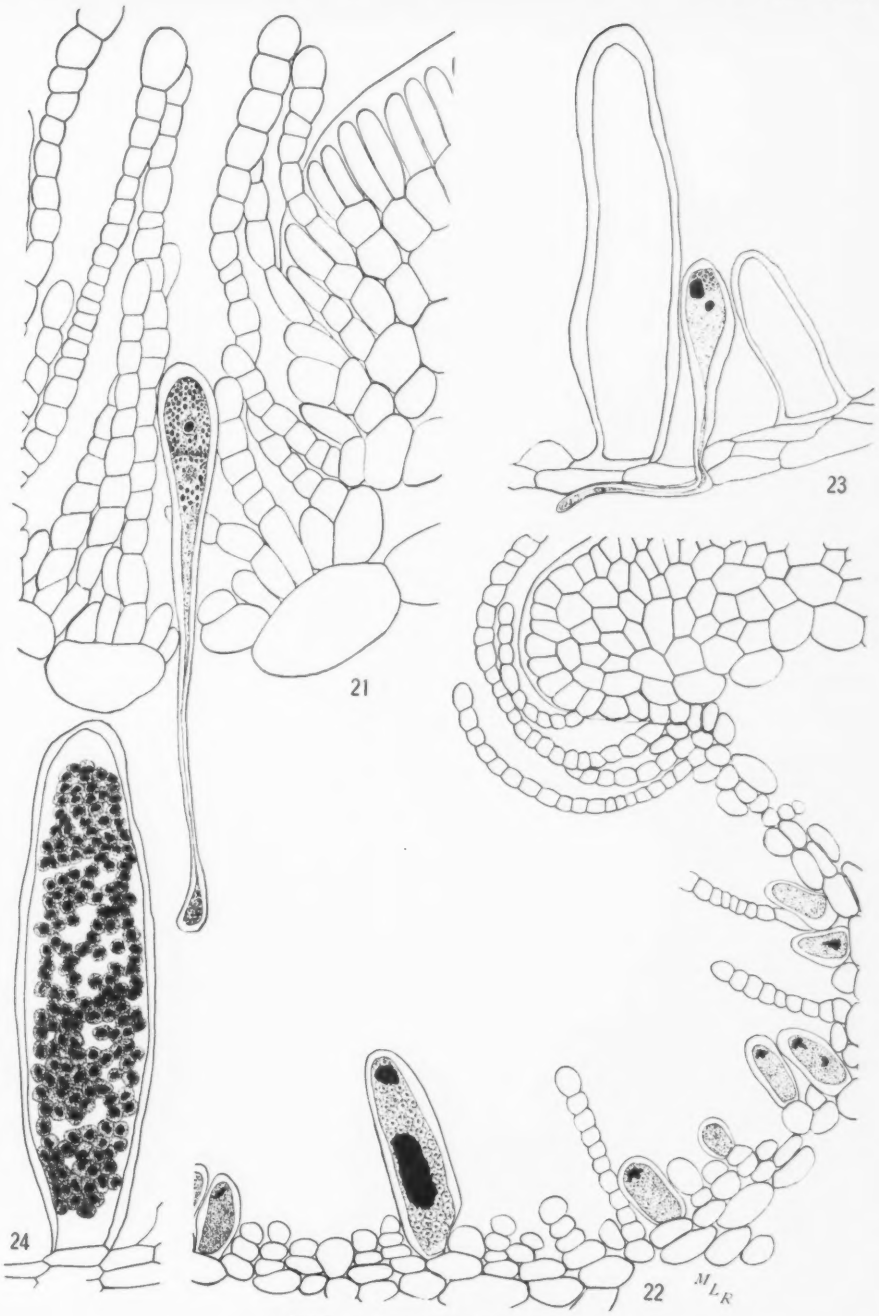


ROE on SPLACHNIDIUM

THE RELATION BETWEEN OXIDASE AND CATALASE IN PLANT TISSUES

G. B. REED

(WITH ONE FIGURE)

In a previous paper[1] the writer has pointed out that although substances which act as oxidases or peroxidases usually decompose hydrogen peroxide, and although many authors think that there is a causal connection between the two processes, yet in the case of colloidal platinum they are quite independent. The question then arises, to what extent does this apply to the living cell? This is of particular interest in view of the fact that some investigators suppose that oxidase action depends in some way upon the activity of a catalase.

A few instances are on record in which, by some special treatment, plant oxidases have been prepared so as to give no catalase action. LIEBERMANN[2] found that by shaking an extract of malt with mercuric oxide and magnesia, or by heating the extract to 80° C., its catalase activity was destroyed but it still exhibited some peroxidase action. LOEW[3] reports that after treating an aqueous extract of fresh tobacco with one-fifth its volume of absolute alcohol it had no action on hydrogen peroxide, but still activated directly the oxidation of gum guaiac. Finally, KASANSKI[4] has shown that the catalase action of certain plant and animal extracts may be destroyed by the addition of strong solutions of pyrogallol or sugar, without completely inhibiting their peroxidase action.

More conclusive evidence that peroxidase action is independent of any ability to decompose hydrogen peroxide was obtained by the writer from a study of pineapple extracts. Pineapple juice always contains very active peroxidases; catalase reactions were

[1] REED, G. B., BOT. GAZ. **62**:233–238. 1916.

[2] LIEBERMANN, P., and L., Pflüger's Archiv. **108**:489–495. 1905.

[3] LOEW, O., Report no. 68. U.S. Dept. Agric., p. 47. 1901.

[4] KASANSKI, Biochem. Zeit. **39**:64–72. 1911.

only obtained, however, under certain conditions which are concerned with the stage of development of the fruit.

After some preliminary experimentation, 3 fruits of the same variety were selected. One was quite ripe and soft; another, although it had turned yellow, was still rather hard; the third had only partly lost its green color. The juice of each of these fruits was pressed out separately in a mortar, filtered, and the catalase activity determined by mixing 2 cc. of the juice in each case with 100 cc. of 0.05 M hydrogen peroxide and measuring the pressure of the oxygen evolved. For this purpose the hydrogen peroxide was placed in a 300 cc. bottle which was provided with a 3-hole stopper carrying a small separatory funnel in which the juice was placed, a paraffin manometer and a tube, closed by a stopcock, for the equalization of the initial pressure. The whole apparatus was submerged in a tank of water with a glass wall, the temperature of which was kept constant. After all the solutions had reached the same temperature the stopcock of the funnel was open and the juice allowed to run into the peroxide. On closing the stopcock of the funnel, as well as that of the pressure tube which had previously been open, the reaction was started

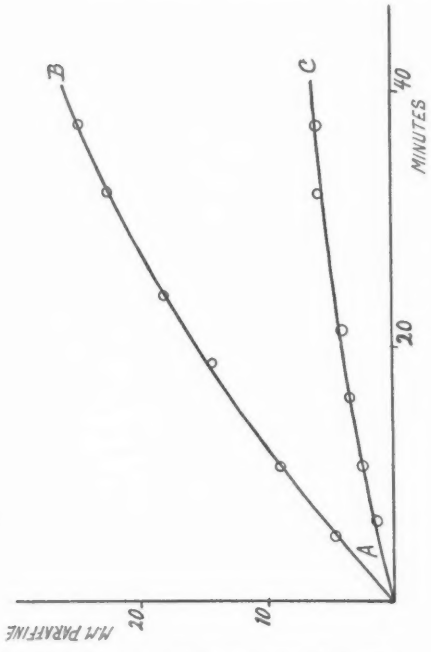


FIG. 1.—Curves representing decomposition of hydrogen peroxide: upper curve *AB* represents action of an extract of a well ripened pineapple; lower curve *AC* represents action of an extract of a partially green pineapple; ordinates represent pressure of oxygen produced (expressed in mm. of liquid paraffin in a paraffin manometer); abscissae represent time in minutes.

at atmospheric pressure.[5] The glass wall of the tank allowed easy reading of the manometer.

The curve *AB* (fig. 1) shows the rate of decomposition of the peroxide (as shown by the manometer readings) with the juice of the ripe fruit. Comparing this with *AC* of the same figure, which represents the rate of decomposition by the juice of the partially ripe fruit, it will be apparent that the latter has much less catalase activity than the former. Moreover, the juice of the green fruit showed no action on this concentration of hydrogen peroxide, or on a stronger solution, whether measured by the pressure method or by simply observing the evolution of bubbles of gas. As might be expected from these results, different fruits exhibit great variation in their catalase activity, but in every case the juice of those in a more or less green condition showed no action in decomposing hydrogen peroxide.

All of these fruits, however, showed approximately the same peroxidase activity, as the following determinations indicate. From each kind of fruit 10 cc. of juice was mixed separately with 100 cc. of 2 per cent pyrogallol solution containing 0.05 M hydrogen peroxide, placed in open beakers and maintained at 18° C. for two hours. The purpurogallin formed from the oxidation was then filtered off, dried, and weighed, after the method of BACH and CHODAT.

Comparing these results, stated in table I, with the previous measurements on the rate of hydrogen peroxide decomposition by

TABLE I

AMOUNT OF PURPUROGALLIN FORMED IN OXIDATION OF 100 CC. OF 2 PER CENT PYROGALLOL CONTAINING 0.05 M HYDROGEN PEROXIDE AND 10 CC. OF EXTRACT

Peroxidase from	Gm. purpurogallin formed in 2 hours
Ripe pineapple	0.986
Partially ripe pineapple	1.020
Green pineapple	1.096
Control; boiled pineapple	0.081

[5] The simpler method of titrating the unused hydrogen peroxide with permanganate could not be satisfactorily employed under these conditions, since the extract of the ferment had a reducing action on the permanganate.

the same extracts, it is evident that the peroxidase activity of pineapple juice is not dependent upon the rate of decomposition of hydrogen peroxide.

From the writer's studies on the behavior of platinum black (*loc. cit.*), in which it was shown that factors which influenced the catalase action have no effect on the oxidative activity, and from the fact that in certain stages of their development pineapples contain oxidases but no catalase, we may conclude that the substances which effect the decomposition of hydrogen peroxide are not of necessity concerned with the enzymes which accelerate peroxide oxidations. It may be added that the fact that catalase is not universally present in living cells, as LOEW and others suppose, has considerable theoretical interest.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

BRIEFER ARTICLES

A NEW HYBRID COLUMBINE

The year before last my wife crossed *Aquilegia chrysantha* Gray (a garden strain) with *A. desertorum* (Jones) Ckll. from Santa Fé Canyon, New Mexico. The hybrids have bloomed this year, and are found to present some interesting characters. In order to make these clear, it is first necessary to indicate the principal diagnostic features of *A. desertorum*.

Aquilegia desertorum (Jones) Ckll.—WOOTON and STANDLEY, in their *Flora of New Mexico*, do not distinguish *A. desertorum* from *A. elegantula* Greene. Direct comparison of living plants has not yet been possible, but *A. elegantula* is properly a forest-loving species of higher altitudes, essentially a mesophytic plant. *A. desertorum*, as its name indicates, is xerophytic, living on rocky slopes in the transition zone, and is remarkable for its enormous root and long life. The early leaves have a strong red suffusion, and the plant comes into flower long before the other Rocky Mountain species, so that only a few belated blossoms can be obtained for crossing with *A. chrysantha* or *A. caerulea*. As I write (June 1), *A. desertorum* has still one or two flowers, while the first flower of *A. chrysantha* is opening. *A. caerulea* is in full flower, however, coming between the periods of *A. desertorum* and *A. chrysantha*. The following description is from a characteristic flower of *A. desertorum*.

Sepals short, about 9 mm. long, 3.5 mm. broad, lanceolate, reddish or partly pale yellowish, with purplish tips; upright, and extending about 2 mm. beyond ends of petals. Petals 25 mm. long (to end of spur), spurs only moderately divergent; about apical 5 mm. of petals light yellow, the rest bright scarlet; this scarlet appearance is wholly due to an anthocyan pigment, which looks pink by transmitted light, and readily passes into solution in weak nitric acid; with liquor potassae it first turns lilac, and then gives the usual green reaction; the lilac can be observed under the microscope fringing the edge of the alkaline fluid. Stamens exserted 7 mm. beyond ends of petals; anthers yellow. Pistils exserted 4 mm. beyond stamens.

Aquilegia desertorum × *chrysantha*, new hybrid.—The early leaves showed more or less distinct reddish or purple color, which is wholly

wanting in *A. chrysantha*. The time of flowering is intermediate between that of the two parents; the plants were in full flower by the second half of May. The flowers are much broader than those of *A. desertorum*, with relatively short spurs (that is, in relation to the breadth), which is not a character of either parent. The sepals are strongly divergent, a character of *A. chrysantha*. The following description is from a characteristic flower.

Flower nodding. Sepals about 19 mm. long and 8 mm. broad, pink, with a faintly purplish shade, or the apex distinctly purplish. Petals 30 mm. long (to end of spur), 8 mm. wide near apex, broadly truncate and sub-marginate apically; apical 10 mm. cream color, spur rose-pink; spurs much broader basally than in *A. desertorum*.

Two plants, representing each parent as seed-producer, are essentially alike; but a third, recorded as from *A. chrysantha* pollen on *A. desertorum*, is distinctly different, having longer spurs (petals 34 mm., the truncate apex 7 mm. wide), and longer, narrower sepals (21 mm. long, 6 mm. wide), while the color of the spurs is more purplish, owing to less acidity. It is just possible that this represents *A. caerulea* pollen on *A. desertorum*, due to some unobserved insect, although the heads were covered and the records do not indicate any such cross as having purposely been made.

Thus it appears that the color of the flowers and the time of flowering of the F_1 hybrid are clearly intermediate; but the form of the flowers departs from both parents in the direction of the *A. vulgaris* group. The form of the flower, however, may be given a simple Mendelian interpretation, if we say that the dominant characters are the spur-length of *desertorum* and the flower-width of *chrysantha*.

We also have a row of *A. chrysantha*×*A. caerulea* hybrids, but this form has long been known in gardens. It blooms with *A. caerulea*, and has the flowers paler than *A. caerulea*, with the white replaced by light yellow, which fades to white as the flowers grow old. On the whole, it is nearly *A. caerulea*, with the yellow of *A. chrysantha* superimposed upon it.—T. D. A. COCKERELL, *Boulder, Colorado.*

AN ALTERNARIA ON SONCHUS

(WITH ONE FIGURE)

During recent studies on *Alternaria* and *Macrosporium* much material was sent to me by various botanists. Among this was an *Alternaria* collected by Dr. DAVIS near Madison, Wisconsin, which

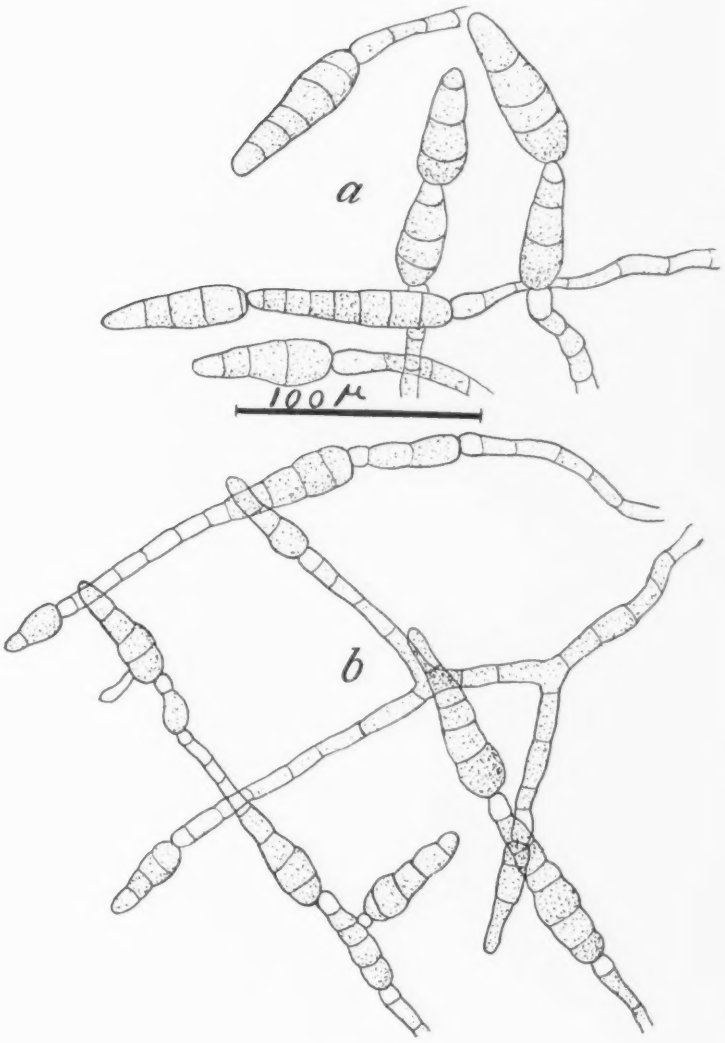


FIG. 1

caused spotting of the leaves of *Sonchus asper*. It is quite different, in so far as I have been able to discover, from any described species.

Pure cultures were obtained from spots on *Lactuca canadensis*, produced by means of crude inoculations with fragments of diseased leaf tissue of *Sonchus asper*. Spots were consistently produced on *Lactuca canadensis* by spores of the fungus from pure culture, and were not in any essential feature different from those occurring naturally on *Sonchus asper*. The spots enlarged rapidly (2–3 cm. in diameter in 10 days), the parasitism apparently being vigorous. On cultivated lettuce and dandelion small flecks were produced by the spores on plants kept under bell jars, but on these hosts the fungus failed to maintain itself. This species was the only one under study which failed to grow at 30° C., although at 20° C. growth was vigorous. The description submitted to me by Dr. J. J. DAVIS is as follows:

Alternaria Sonchi Davis, sp. nov.—Spots definite, orbicular to irregular, brown to cinereous, usually with a narrow dark margin above, darker and immarginate below, 0.5–1 cm. in diameter; conidiophores hypophyllous, effused, straight or slightly geniculate, cylindrical, obtuse, dilute brown, apical portion nearly hyaline, 18–55×7–8μ; conidia obclavate with obtuse apex, 5–8-septate, second, third, or first cells, one or all, occasionally divided by a vertical or oblique septum, 80–110×18–20 μ, borne singly or in chains of 2 or 3 spores.—JOHN A. ELLIOTT, *Laboratory of Plant Pathology, University of Illinois.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Photosynthesis.—We are indebted to WILLSTÄTTER and his co-workers[1] for a series of contributions on the chemistry of the chloroplast and the mechanism and behavior of the photosynthetic apparatus. It is a promising sign that so eminent a chemist as WILLSTÄTTER recognizes that the chemistry of the cell is not the chemistry of the test tube and flask of the "true chemist," but rather the chemistry of a highly complex colloidal substrate with its enzymes. Physiologists can well look forward with keen anticipation to further notes from his laboratory, since we have this statement concerning his future work: "Analyse physiologisch bedeutsamer Stoffe ist nicht Selbstzweck, sondern Vorbereitung für physiologische Forschung."

The leaf pigment chlorophyll is a mixture of two green pigments, a blue-green (chlorophyll *a*) and a yellow-green (chlorophyll *b*), and two yellow pigments, carotin and xanthophyll. In the Phaeophyceae a third carotinoid (fucoxanthin) is found. The chlorophylls of all plants investigated (almost 200) are neutral substances carrying a complex magnesium compound whose molecule is built up of 4 freely substituting pyrrol nuclei and are of the following compositions respectively: $(C_{31}H_{29}N_3Mg)$ (CONH) $(COOCH_3)$ $(COOC_{20}H_{39})$ and $(C_{31}H_{27}ON_3Mg)$ (CONH) $(COOCH_3)$ $(COOC_{20}H_{39})$. These compositions were determined by a judicious and cautious treatment of chlorophyll extracts with proper alkalies and acids in proper concentrations. It was found that splitting with acid made it possible to preserve and find in the products of hydrolysis that compound which is separated by alkalies, and, conversely, that the alkali derivatives exhibited a characteristic group which is destroyed with extraordinary ease by acids. Saponification of the pigment with alkali yields green, water soluble salts, the chlorophyllins. Progressive removal of

[1] WILLSTÄTTER, R., and STOLL, A., Untersuchungen über Chlorophyll. 8vo. pp. viii+424. *pls. 11.* Berlin: Julius Springer. 1913; see review of recent work on the pigments of the green leaf and the processes connected with them in New Phytol. **14**:281–294. 1915.

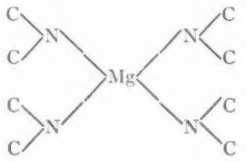
———, Über die chemischen Einrichtungen des Assimilationsapparates. Sitzb. Kgl. Preuss. Akad. Wiss. **20**:322. 1915.

———, Über die Assimilation ergrünender Blätter. Sitzb. Kgl. Preuss. Akad. Wiss. **36**:523. 1915.

———, Untersuchungen über die Assimilation der Kohlensäure. Ber. Chem. Gesells. **48**:1540–1564. 1915.

WILSTÄTTER, R., Sitzb. Kgl. Preuss. Akad. Wiss. **32**:484. 1915.

the carboxyl groups with alkali yields a series of phyllins, the last oxygen free one being aetiophyllin, which carries the Mg group intact, has the composition $C_{31}H_{34}N_4Mg$, and hypothetically the structural nucleus



Acid treatment of the phyllins removes the Mg and yields the porphyrins, aetioporphyrin ($C_{31}H_{36}N_4$), the equivalent of aetiophyllin, being the last of the series.

Treatment of chlorophyll with acids attacks another group, no saponification takes place, and a waxlike Mg-free chlorophyll derivative, phaeophytin, is obtained. Its solution differs in color from that of chlorophyll, but the green returns upon introduction of a metal, such as Zn, Cu, or Mg. Saponification of phaeophytin with alkali yields nitrogen-containing acids and a nitrogen-free alcohol phytol ($C_{20}H_{39}OH$), which may constitute one-third of the weight of chlorophyll and varies widely in amount in various chlorophylls. Chlorophyll is associated with an enzyme, chlorophyllase, active in alcoholic media, which causes an alcoholysis of the pigment and yields the chlorophyllides, the crystalline chlorophyll of the literature, according to the equation

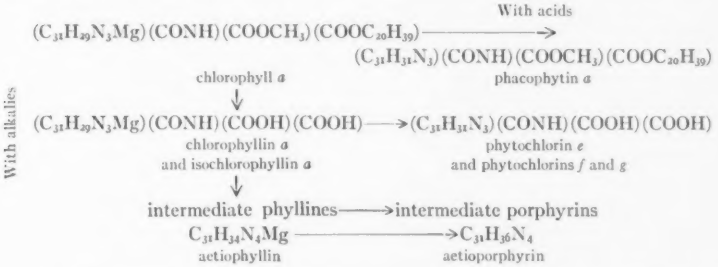
$$\underset{\text{chlorophyll } a}{(C_{32}H_{30}ON_4Mg)\ (COOCOC_3)\ (COOC_{20}H_{39})} + C_2H_5OH \rightarrow \underset{\text{phytol}}{C_{20}H_{39}OH} +$$

$$\underset{\text{ethyl chlorophyllid } a}{(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_2H_5)}.$$

Plants can be divided into chlorophyllase-rich, such as *Heracleum Spondylium*, and chlorophyll-rich, such as *Urtica* sp.

The substance originally named phaeophytin by WILLSTÄTTER always yielded two distinct groups of decomposition products, one olive green in solution, the phytochlorins, the other red, the phytorhodins. Phytochlorin *e*, typical of the former series, is a tricarboxylic acid with two free carboxyl groups and one bound as a lactam; while phytorhodin *g* is a tetracarboxylic acid, only two or three of the carboxyls being in the free state. They are separated by a method based upon their different solubilities in ether and hydrochloric acid. Determination of the molecular weight of these substances, their occurrence in definite weight proportions, and their non-interconvertibility convinced WILLSTÄTTER that phytochlorin and phytorhodin arise from separate and distinct phaeophytins (*a* and *b* respectively), and that these in turn are derived from two distinct chlorophylls (chlorophyll *a* and *b*). These chlorophylls are obtainable in microcrystalline form and differ somewhat in their solubilities in various reagents.

The extraction of the chlorophylls can be carried out with dried or fresh material and is based on the use of solvents containing a moderate content of water, the latter forming salt solutions with some of the cell contents which alter the condition of the chlorophyll and render it soluble in the organic solvent. Separation and purification of chlorophyll *a* and *b*, carotin, and xanthophyll is based on their differing solubilities in solvents. Little is known of the chemistry of the carotinoids. They are hydrocarbons, both give non-fluorescent solutions, stable toward alkali but easily dissociated by acids, and both undergo auto-oxidation. Carotin and xanthophyll have the formulae $C_{40}H_{56}$ and $C_{40}H_{56}O_2$ respectively.



Willstätter and Stoll reinvestigated the question of the relation of chlorophyll content to photosynthetic activity of leaves in normal, chlorophyll-poor, vernal, and autumnal leaves. They used a modified Kreussler method and determined the assimilation number, $\frac{CO_2 \text{ assimilated in 1 hour (in gm.).}}{\text{chlorophyll (in gm.)}}$ They find not only that chlorophyll is absolutely essential for photosynthesis, but also that it acts in conjunction with a second indispensable inner factor, an enzyme, which has its seat in the contact zone between plastid and plasma. If the chlorophyll suffers more because of external factors, the assimilation number rises; if the enzyme suffers more, the number falls. The enzyme occurs in greatest proportion in juvenile, greening, etiolated leaves and in yellowish green leaves, and in all of these illumination is the limiting factor, while temperature is the limiting factor in chlorophyll-rich leaves in the photosynthetic process. Conclusions as to various steps in assimilation are drawn from the behavior of isolated chlorophyll as well as non-illuminated leaves toward carbon dioxide. Chlorophyll is not constantly decomposed and regenerated, as asserted by Stokes, neither do chlorophyll *a* and *b*, nor carotin and xanthophyll change into each other to any extent, as assumed originally by Willstätter. The ratio $Q\frac{a}{b}=3$ is about constant under all conditions, though the ratio $Q\frac{c}{x}$ falls during the course of extended experiments. A striking difference was noted in the behavior of chlorophyll towards CO_2 when

in true solution in ether or alcohol, and in colloidal solution, as watery acetone. The former is exceedingly stable toward alkali and acids, while the latter is stable toward alkali, but so sensitive to acids that CO_2 splits it readily into $Mg(H\,CO_3)_2$ and phaeophytin ($C_{55}H_{72}O_5N_4Mg + 2CO_2 + 2H_2O \rightarrow Mg(H\,CO_3)_2 + C_{55}H_{74}O_5N_4$). Such a solution loses half of its metal in 4 days when treated with 5 per cent CO_2. Before the Mg is displaced, however, an intermediary dissociable compound is formed, maximum in quantity at low temperatures. The time factor is much more active in the chlorophyll *a* than in the other compound. Leaves are thus endowed with a mechanism for storing CO_2 much as the blood is. Instead of the albumin of the serum holding the CO_2, however, it is possible that carbamino compounds of amino acids or proteins are involved here. This dissociable CO_2 chlorophyll compound may take up the light energy and give an isomer of greater energy content which is suitable for spontaneous decomposition. This may be the substance that is split enzymatically.

Interesting in this connection is a paper by SPOEHR.[2] In an excellent critical review of the literature he points out certain facts that seem to weigh heavily against the theory of formaldehyde as an intermediate product in photosynthesis and in favor of formic acid as such. Discussing the work of LOEB with CO_2 and silent discharge, USHER and PRIESTLY, BERTHELOT and GUADECHOW, and STOCKLASA with CO_2 and ultraviolet light, he concludes that we have no clear record of a reduction of carbonic acid on its salts to formaldehyde which is applicable to the process going on in the plant leaf or is compatible with conditions met in the leaf. NEF'S success in condensing formaldehyde to sugar might be of significance, he believes, if an enzyme could be substituted for the strong alkali and high temperature used. WILLSTÄTTER'S work shows clearly that enzymes and the colloidal state of chlorophyll and the plasma are important factors in this process. It also shows that the greatest progress in physiology is to be made by work that combines chemical and physiological technique judiciously. SPOEHR finds that while formaldehyde yields sugars in light or dark only in the presence of relatively strong bases, such as do not exist in the plant, CO_2 in aqueous solution in ultraviolet light produces formic acid as the only product, and exposure of this to sunlight or ultraviolet light gives a substance having several properties and the nutritive value of sugars. He believes formic acid may well be the intermediary product of photosynthesis, as suggested by ENGELMANN, especially since organic acids in sunlight give rise to formaldehyde and vice versa. The formaldehyde reported in green leaves, as shown by WAGER and WARNER, may be due to the activity of light on chlorophyll and other plant substances. SPOEHR feels that the findings of GRAFE, BOKORNY, and BAKER, that formaldehyde is assimilated by the green leaf only in presence of sunlight, introduces an entirely new

[2] SPOEHR, H. A., The theories of photosynthesis in the light of some new facts. Plant World **19**:1–16. 1916.

factor into the BAEYER hypothesis, to which altogether too much attention has been paid by chemists and physiologists.

The reviewer feels that the results of the chemist WILLSTÄTTER, who, to paraphrase a statement of JOHANNSEN'S, carries on his physiological studies not as chemistry, but with chemistry, deserve the notice of all physiologists and chemists, and are full of promise of a new attack upon the most fundamental material problem of mankind, namely, the increase of the food supply of the world.—G. K. K. LINK.

The bubble method in photosynthesis.—KNIEP[3] has made a critical study of the value of the bubble method for comparing the rate of photosynthesis in water plants under various conditions. From his work it is evident that the method must be applied with great caution. Part I deals with the percentage of oxygen in eliminated gas bubbles. This percentage rises as the rate of bubbling increases, but not in direct proportion. If one were to find the total oxygen production due to photosynthesis, he would need to determine (1) the volume of the eliminated gas with its percentage of oxygen, and (2) the amount of oxygen that diffused into the bathing water. For the analysis of eliminated gas KNIEP used Krogh's apparatus, by which the percentage of oxygen and carbon dioxide can be determined in very small samples of 4–6 mm.^{3} The amount of oxygen diffusing into the bathing water was determined before and after a period of photosynthesis by WINKLER'S titration method. The percentage of oxygen in the eliminated gas varied from 22.8 per cent in slow photosynthesis to 45 per cent in rapid photosynthesis.

The whole matter is complex. The oxygen production in photosynthesis not only increases the volume of gas in the intercellular spaces and leads to elimination of gas from the cut surface of the stem, but it increases the oxygen percentage in these spaces and thereby steepens or sets up diffusion gradients (falling gradient of oxygen from intercellular spaces to bathing water and of nitrogen in the opposite direction). Carbon dioxide diffusion gradients due to its consumption must be considered also. Hence the amount of gas given off as bubbles and its richness in oxygen and other gases is the resultant of all these processes acting together, a given set of conditions giving one equilibrium and a change in conditions gradually leading to another set of conditions.

Part II presents the influence of water movement upon the bubble outgo. With 1 per cent $KHCO_3$ in distilled water as the bathing medium, water movement temporarily stops the bubble outgo. With tap water (supersaturated) the outgo of bubbles increases and continues in darkness even in dead plants. This behavior is all explained on the basis of the effect of the water movement upon the gas gradients. On the basis of these results KNIEP pointed out the

[3] KNIEP, HANS, Über den Gasaustausch der Wasserpflanzen. Ein Beiträge zur Kutik der Blazenzählmethode. Jahrb. Wiss. Bot. **56**:460–509. 1915. PFEFFER'S Festschrift.

probable errors in the use of the method for studying the rate of photosynthesis as affected by carbon dioxide concentration.

Part III deals with the effect of short periods of darkening of varying length upon the time needed for resumption of bubbling after reillumination. The longer the darkening, within certain limits, the longer the time required for bubble resumption. This is explained by diffusion (during darkening, as determined by the gas gradients) contracting the gas volume in the intracellular spaces and thereby drawing water into these spaces. The longer the period of darkening the greater the contraction. On the basis of these results KNIEP develops in part IV a method for determining the minimum light intensity necessary to produce oxygen elimination into the intracellular spaces, or to give an excess of photosynthetic gas exchange over the respiratory exchange. It is 2.8 candle meters. This paper deals mainly with methods that are to be used later in answering various questions in photosynthesis.—WILLIAM CROCKER.

Life histories in Laminariaceae.—In his recent investigation of *Saccorhiza bulbosa*, SAUVAGEAU[4] found that germinating zoospores behave quite at variance with results reported by other workers. Instead of a protonema, as found by WILLIAMS,[5] DREW,[6] and KILLIAN,[7] he found that the erect plant is developed directly, and also[8] that *Saccorhiza* shows heterogamy and distinct alternation of generations.

After swimming about for a time, the zoospore comes to rest, rounds out, and develops a membrane, becoming an "embryospore." Subsequent behavior determines whether the embryospore is to be a male or female gametophyte. In the latter case the embryospore doubles its diameter, multiplies its chromatophores without division of the nucleus, and in a short time a tube puts out, of about the same diameter as the embryospore, though its length may greatly exceed the diameter; into the tip of this tube the entire contents of the embryospore migrate. Finally the membrane is ruptured and there escapes a single naked uninucleate mass, slightly elongated in form, which remains attached to the mouth of the empty embryospore tube. This naked mass, which is the

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[4] SAUVAGEAU, C., Sur le développement et la biologie d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **160**:445–448. 1915; **161**:740–742. 1915.

[5] WILLIAMS, J. L., Germination of the zoospore in the Laminariaceae. Rept. British Ass. Adv. Sci. (Bradford) 1900.

———, The zoospores of the Laminariaceae and their germination. Rept. British Ass. Adv. Sci. (Dundee) 1912.

[6] DREW, G. H., The reproduction and early development of *Laminaria digitata* and *L. saccharina*. Ann. Botany **24**:177–190. 1910.

[7] KILLIAN, K., Beiträge zur Kenntnis der Laminarien. Zeitschr. Bot. **3**:433–494. 1911.

[8] SAUVAGEAU, C., Sur la sexualité hétérogamique d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:796–799. 1915.

egg, soon develops a wall about itself, separating it from the abandoned tube. Sometimes the embryospore nucleus divides, giving two cells; then the anterior cell behaves like an ordinary embryospore; or if a monosiphonous filament of several uninucleate cells is formed, the anterior cell again functions. Only in weak and poorly developed cultures did SAUVAGEAU observe erect branches from the embryospore; in these cases each erect branch behaves in the manner described.

If the embryospore is to develop a male gametophyte, its diameter is increased little or none, elongation is very rapid, but the entire structure remains very minute. The antheridia develop as rounded colorless buds, each enclosing a single sperm, which after its escape is seen to be motile, colorless, and laterally biciliate.

Fertilization was not observed, but SAUVAGEAU assumes that it occurs and that from the fertilized egg the plantlet or sporophyte develops immediately. His account from this stage on differs in few essentials from the accounts of other workers, the stipe and blade being differentiated at a very early stage.

It is to be regretted that at the critical stages details are not more clearly given. However, it is evident from this account, as also from that of DREW, that there are certain phases in the life history of the Laminariaceae which are in need of critical investigation, especially from a cytological point of view. DREW concluded that as a result of conjugation of isogamous gametes a filament (the sporophyte) developed which in turn gave rise to the plantlet (the gametophyte). SAUVAGEAU concludes that the gametophyte comes very early in the cycle; in fact, it is practically a walled zoospore, and the plantlet which is produced as the result of fertilization of the egg is the sporophyte. It would seem that a careful investigation might clear up the haziness which still remains about these phases in the life history.—MABEL L. ROE.

Tannins.—MOELLER[9] has noted peptization phenomena in tannin solutions, and believes that they form the basis of the process of tanning. A tannin extract contains two sorts of substances: (1) a tannin that is soluble in water, and (2) an accompanying substance, a gel, which is insoluble in water. With pyrogallol tannins this accompanying substance is called ellagic acid, and with pyrocatechin tannins it is called phlobaphene. The ellagic acid and phlobaphene, while insoluble in water, are soluble in the tannin solution; that is, the tannin peptizes the ellagic acid gel and the phlobaphene gel, the tannin being called the peptizator and the gel the peptized substance. In the process of tanning the hide adsorbs the peptizator and the gel coagulates out, surrounds the fibers of the hide, and leather is formed. He says a true solution cannot tan a hide, but only a peptized solution, and that all tanning solutions

[9] MOELLER, W., Die Peptisationserscheinungen in Gerbstofflösungen. Kolloid. Zeitsch. **16**:69–76. 1915.

contain a gel held in solution as a hydrosol by the action of a peptizator. Purified tannin solutions do not tan, and the more "impure" the solution the better the tanning effect. Watery tanning solutions on standing in the air undergo changes that increase their tanning value, owing to the formation of an insoluble product that becomes peptized.

MOELLER finds a similar situation exists in the artificially prepared tanning solutions. In chinon tanning solutions the chinon takes the place of a peptizator, while hydrochinon is the peptized gel. In the mineral tanning solutions an insoluble metallic hydroxide is peptized into the solution condition by the peptizing action of the acid salt solution. According to MOELLER'S view, the properties of a tanning extract depend upon the equilibrium between the peptizator and the peptized substance, and the character of the leather depends upon the nature and quantity of the peptized gel that is taken up by the hide fibers.—F. E. DENNY.

Taxonomic notes.—Miss GILKEY[10] has published a revision of the Tuberales of California, preceded by an account of the distribution of truffles in California, their economic importance, and their morphology and phylogeny. Accompanied by very full discussion, 11 genera are presented, including 32 species, the largest genus being *Tuber*, with 12 species. A new genus (*Hydnotryopsis*) and 15 new species are described, distributed among the following genera: *Hydnocystis*, *Genea* (3), *Hydnotrya*, *Tuber* (6), *Piersonia*, *Geopora* (2), and *Hydnotryopsis*.

WEST[11] has described a new genus of mycorrhizal fungi associated with the roots of the various genera of Marattiaceae. He names it *Stigeosporium*, and regards it as of special interest inasmuch as it produces "under natural conditions distinct reproductive bodies within the tissues of the host root."

WILLIAMS[12] has published a list of Peruvian mosses based upon two collections. It includes 71 species, among them 6 new species in the following genera: *Leptodontium*, *Globulina*, *Tortula*, *Grimmia*, *Bryum*, and *Hygrohypnum*.—J. M. C.

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[10] GILKEY, HELEN MARGARET, A revision of the Tuberales of California. Univ. Calif. Publ. Bot. **6**:275–356. *pls. 26–30.* 1916.

[11] WEST, CYRIL, *Stigeosporium Marattiacearum*, gen. et sp. nov. Ann. Botany **30**:357. 1916.

[12] WILLIAMS, R. S., Peruvian mosses. Bull. Torr. Bot. Club **43**:323–334. *pls. 17–20.* 1916.